Factors Affecting Wheat Nutritional Value for Broiler Chickens

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ABSTRACT Wheat is the main energy source in European broiler diets. Although many studies have been developed about its nutritive value, its accurate prediction and utilization by broilers is not elucidated yet. This thesis deals with the factors that affect wheat nutritive value for broiler chickens: physico-chemical variability in wheat grains, the use of NSP-degrading enzymes, wheat cultivar and origin, and nitrogen fertilization to wheat crop. Broilers (21 to 30 d of age) were offered complete diets including wheat samples at 55 or 70% from different origin and grown under different conditions. Ileal and fecal starch digestibility, rate of starch digestion, nitrogen-corrected metabolizable energy and animal performance were measured. Results suggested that starch and crude protein were the main variables affecting in a positive and negative way, respectively, the nutritive value of wheat for broiler chickens. The different factors studied in wheat samples influenced both physico-chemical properties and nitrogen-corrected metabolizable energy of the wheat but not ileal and fecal starch digestibility. The use of NSP-degrading enzymes increased nutrient digestibility and nitrogen-corrected metabolizable energy but did not eliminate the differences among wheat cultivars. Subsequent experiments demonstrated that the increase in crude protein content of the wheat by crop nitrogen fertilization had no effect on rate of starch digestion. Wheat samples differed in their starch digestion rate but they could not be classified based on the name cultivar alone because cultivar origin also affected starch digestion rate. Animal performance was affected by the rate of wheat starch digestion and there was a narrow range where animal performance was maximized. It was concluded that the variability in the nutritive utilization of wheat is mainly affected by its starch digestion rate more than by its total starch digestibility.

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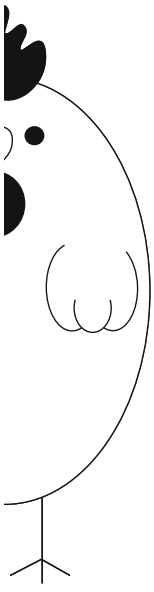
CHAPTER 1

General Introduction



Modern farm chickens can grow up to 2.5 kg in 42 days after hatching. This enormous capacity for growth must be enabled by a well defined and completely balanced diet. A balanced diet is a mixture of feedstuffs that provides macro- and micro-ingredients to cover the maintenance and growth requirements of the animals. In addition, the diet should protect against the potential pathogen microorganisms which are present in the animal's gut.
For broiler chickens the macro-ingredients are usually corn and wheat (cereals), soya and sunflower oil (vegetable oils), and soya beans (oil seeds). In European broiler diets, wheat is used to a greater extent than corn because it is cheaper and does not contain xantophylls which pigment the meat. As a consequence, wheat is the main cereal used in Europe for broilers where it can represent around 50-55% of the total apparent metabolizable energy **(AME)** and around 35-40% of the protein given to the broiler in the feed (Wiseman et al., 2000).
The relevant wheat crops (for commercial animal nutrition) are limited to two species of the genus *Triticum; Triticum aestivum and Triticum turgidum.* Of these, *Triticum aestivum*, commonly recognize as "soft wheat" is by far the most widely used. Morphologically, wheat grain can be divided into three components – the bran (15-17% of the grain, including the aleurone layer), the endosperm (80-85% of the grain) and the germ (2-3% of the grain) (Morrison, 1993). Chemically, on dry matter **(DM)** basis, wheat is composed by 68% starch **(ST)** (ewers), 13% protein, 3% crude fiber, 2% oil and 2% ash (CVB, 2007). The percentage of nutrients present in the different components of the wheat grain is very different among samples but ST and protein are mainly located in the endosperm whereas the fiber and the mineral fractions are mainly located in the bran (Table 1).
Wheats received at the feed mill are normally a mixture of different wheat cultivars stored for an unspecified time (sometimes more than six months) and they are accepted or rejected based on grain to dust percentage and grain weight as main parameters of quality control. In this sense, feed wheat is much more variable than the wheat used for baking. The bakery industry has established a high standard quality control of all the wheat grains received at the mill. Parameters like crude protein content, dough-making properties, α-amylase activity or hagberg falling number are determinants in the final use of a specific wheat and can be a reason for rejection. When a rejection occurs baking wheats are directed to feed mills to be used as feed for animals. Therefore, wheat used for feed purposes can be very variable, not only in terms of storage and the mixture of genetic varieties (cultivars) but also in terms of wheat physico-chemical properties. As a consequence the feed industry faces a difficult and important job. They have to establish properties with regard to the nutritive value of this raw material.

Table 1. Concentration of the nutrients of the wheat in the different components (%). Adapted from Morrison (1999)

	Starch	Crude Protein	Crude Fiber	Lipids	Minerals
Bran (incl. aleurone)	-	20	93	30	67
Endosperm	100	72	4	50	23
Germ	-	8	3	20	10

The feed industry and the scientific world together have conducted several research programs and studies in an attempt to identify and quantify those factor(s) which mostly influence the wheat nutritive value for animal feeding. Most of the research has focused on one or two factors, where the carbohydrate fraction of the wheat is considered of relevance. There are two main reasons for this:

- the non-starch polysaccharide **(NSP)** fraction of the wheat can have negative effects in broiler performance and

- the digestible starch is what contributes most to the apparent metabolizable energy content of wheat.

Wheat NSP are mainly arabinoxylans (average, 6% in DM), ß-glucans (average 0.8% in DM) and cellulose (average 2% in DM). Following the classification of Van Soest (1963a, 1963b) and on DM basis wheat has 12% neutral-detergent fiber **(NDF)**, 4% acid-detergent fiber **(ADF)** and 1% acid-detergent lignin **(ADL)**. The ability of the bird to digest NSP is almost absent as it does not produce and secrete the enzymes for that. Moreover, the soluble fraction of the arabinoxylans (average 1.8% in DM of total arabinoxylans) is known to be the responsible for the increase in viscosity of the digesta inside the small intestine. Viscosity is considered responsible for the negative impact on digestion and also for absorption of nutrients (Choct and Annison, 1990, 1992) being also linked with digestive disorders such as sticky litter and hock burns (Choct et al., 1996). The problems associated with the NSP fraction can be alleviated by the use of NSP-degrading enzymes. Many studies have shown that enzyme preparations with xylanase activity are able to increase the nutritive value of wheat-based diets (Bedford and Classen, 1992;

Choct et al. 1995; Steendeldt et al., 1995) therefore many nutritionists consider the use of NSP-degrading enzymes highly necessary when formulating wheat-based diets. However, there is a lack of knowledge about to what extend then NSP-degrading enzymes can compensate for the negative effect of NSP on the nutritional value of wheat. Especially it is not known if wheat grains of different origin can be made similar in nutritional value by the use of exogenous enzymes.

Starch consists of two polymers of glucose, amylopectin (75% as average) and amylase (25% as average). It is the main energy component of wheat and contributes up to 78% of its AME (Longstaff and McNab, 1986). Therefore, a positive correlation ($r = 0.70$) between ST and AME is expected and it has been reported (Huyghebaert and Schöner, 1999). However, sometimes the utilization of the energy in the wheat by broiler chickens is poor and unrelated to the ST content in the wheat (Rogel et al., 1987). In those cases ST digestibility rather that ST content may explain the lack of relationship. In fact, Rogel et al. (1987) and Carré et al. (2002) found high variability in ST digestibility of different wheat samples (from 82 to 99%). Wiseman et al. (2000) related the ST digestibility of wheat to its AME ($r = 0.96$, $n = 16$). Moreover, a major cause of low-AME values of wheat based diets for broilers is low ST digestibility (Mollah et al., 1983; Wiseman et al., 2000; Svihus, 2001).

However, some intrinsic factors that cause variability in the nutritional value of wheat are not yet completely elucidated. Some wheats that are considered similar and standard with regard to chemical and physical properties still give a large variation in AME and also in animal performance when fed to broiler chickens. In those cases, neither ST content nor ST digestibility correlates well with wheat AME. There is evidence that the rate of ST digestion can affect animal performance (Weurding, 2002). Slowly digestible ST is said to improve animal performance by a better synchronization of ST digestion with protein and amino acid digestion when compared to diets with rapidly digestible ST. Moreover, it seems that animals fed with slowly digestible ST show less *Clostridium Perfringens* (Weurding, 2002) which is a bactaria associated with necrotic enteritis (Craven et al., 1999).

The protein fraction of the wheat can represent up to 40% of the total protein of the diet and therefore, it has to be considered when defining wheat nutritive value. The protein located in the endosperm surrounds the ST granules and interacts with the ST producing a considerable variation in hardness. Extra nitrogen fertilization during growing of wheat increases the amount of protein in wheat, especially the one located in the endosperm (Uhlen et al., 2004). Because 100% of ST is located in the endosperm and embedded in a protein matrix it may be that increasing contents of protein interfere with ST diges-

tion and especially with rate of ST digestion. It has been reported by Svihus and Gullord (2002) that there are negative correlations between protein content and AME. As a consequence, when feeding wheats with a variable protein content also variability in ST digestibility, AME content and animal performance can be expected.
This thesis study has focused on clarifying part of the variation in AME content of wheat. If NSP-degrading enzymes cannot equalize wheat samples it would mean that the viscous NSP fraction of the wheat is not fully responsible for the variability reported. Therefore, other factors have to be studied also. As ST is the main energy supplying nutrient in wheat, ST content itself, ST digestibility and the factors that may influence it are considered in this study. So, the main objective of this study is to evaluate the factors that affect wheat AME and animal performance with special focus on the carbohydrate (mainly ST) fraction of the wheat. For that purpose several factors have been studied.

- First a review has been made which describes the factors that affect wheat AME (Chapter 2).

- The first experiment was designed to determine the variability in physico-chemical properties of wheat as mainly received and used in commercial feed mills and to correlate them with the AMEn of wheat (Chapter 3).

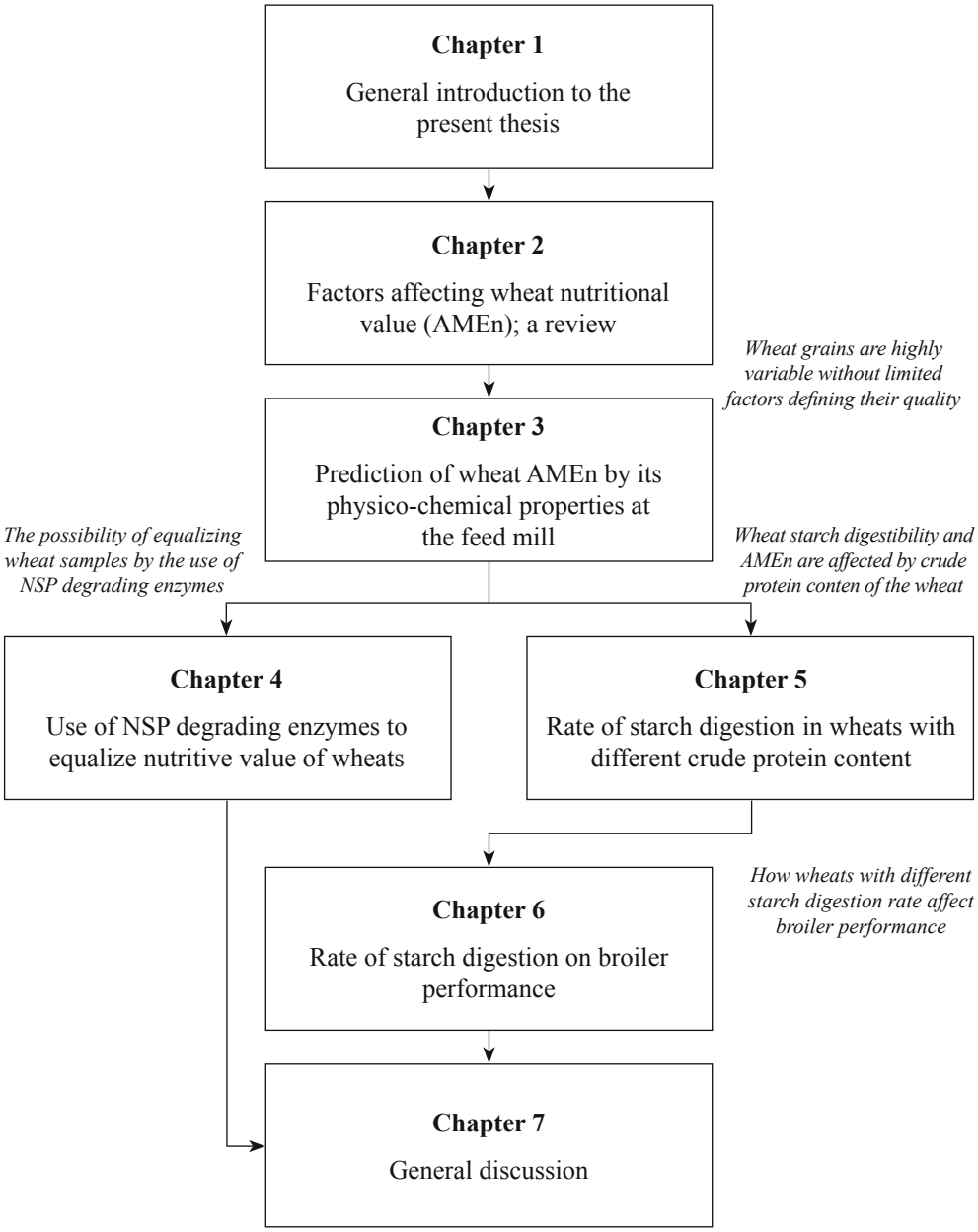
- For the second experiment the effect of the use of enzymes which degrade NSP was studied in different wheat cultivars. The aim was to determine if enzymes are able to minimize the differences in wheat nutritive value (Chapter 4).

- As a follow up of literature the difference in the rate of ST digestion among wheat cultivars with different nitrogen fertilization and as a consequence with different protein content were determined (Chapter 5).

- A fourth experiment was focused on determining the impact of wheat ST digestion rate on broilers performance (Chapter 6).

- In Chapter 7 the results of the different experiments are discussed. An important aspect is how wheat cultivars with similar chemical and physical properties can influence the use of its nutrients in broilers. A conclusion is made on how this knowledge can be used to reduce the variation observed in animals fed wheat-based diets.

Figure 1. A schematic representation of the outline of the thesis



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CHAPTER 2

Variability in Wheat: Factors Affecting Its Nutritional Value

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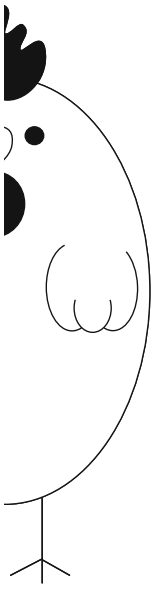

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ABSTRACT Wheat is the main raw material used in Europe as an energy source in broiler diets. Its apparent metabolizable energy **(AME)** and its influence on broiler performance varies among wheat samples. Reasons for that variability can be classified as intrinsic (variety, chemical composition) and extrinsic factors (growing conditions, storage, etc.) that affect nutrient digestibility and availability. However, those factors are not normally considered when formulating the diets for broiler chickens. Moreover, research through the years has questioned the relation between wheat AME and animal performance. This review aims to describe factors that influence the observed variability in wheat nutritive value for broiler chickens by considering origin (variety, growing conditions and post-harvest storage), chemical composition of the grain (carbohydrates and protein) and the broiler chicken.

(Key words: wheat; variety; growing conditions; storage; chemical composition; broiler)

INTRODUCTION

Wheat is an important feed ingredient for poultry diets in Europe. It contributes up to 650 g/kg of diet for finishing broilers. Over 125 million tonnes of wheat per year are produced in the EU (in its 25 member status) and more than 45% of it is used in animal feed (International Grains Council, 2004). Obviously, the high rate of inclusion of wheat in poultry diets can be related to heterogeneous broiler performance, where wheat nutritive value is not well defined and quantified.

It has been reported that apparent metabolizable energy **(AME)** of wheat ranges from 8.49 to 15.9 MJ/kg dry matter **(DM)** (Mollah et al., 1983; Wiseman, 2000; McCracken et al., 2002). Growth performance of broilers which were fed different wheat samples differed as much as 13% (Scott et al., 1998). Moreover there was no correlation between wheat AME and animal performance (Rose and Bedford, 1995; Scott et al., 1998; Steenfeldt, 2001).

There has been a considerable amount of work designed to investigate the reasons for the variability in the nutritional value of wheat. Some have focused on the study of its origin (variety, site of growth, etc.) or physical measurements (storage time, inclusion form, etc.), and their influence on AME (Preston et al., 2001), nutrients digestibility (Preston et al., 2001), gut structure and function (Jones and Taylor, 2001) and animal performance (Jones and Taylor, 2001; Preston et al., 2001). Others have studied nutritive value of wheat as influenced by its chemical composition (crude protein, ether extract, starch, etc.) (Longstaff and McNab, 1986; Pirgozliev et al., 2003). Few studies that comprises both physical and chemical measurements of wheat have been done (Carré et al., 2002; McCraken et al., 2002).

The present paper describes the factors that influence variability in wheat nutritive value for broiler chickens by considering variety, growing conditions, post-harvest storage, chemical composition (carbohydrates and protein) and the broiler chicken.

WHEAT VARIETY

Physical and chemical characteristics of wheat changed according to variety. For example, wheat hardness (Oury et al., 1998; Morris, 2002; Chantret et al., 2005), thousand grain weight (McCracken et al., 2002), viscosity (McNab and Knox, 1999; McCracken et al., 2002), phytase activity (Kim et al., 2002), total NSP (Carré et al., 2002; McCracken et al., 2002), starch content (Bhatty et al., 1974; Swanston et al., 2007) or gross energy content (Bhatty et al., 1974) have been shown to depend on wheat cultivar. However, individual varieties do not respond in a uniform way and even in one variety, energy value is not constant (Wiseman,

2000; Angus and Wiseman, 2003). The reason why this happens is that factors such as harvest year (Waldron et al., 1994; Rose et al., 2001; George and McCracken, 2003; Pirgozliev et al., 2003), harvesting conditions (Zijlstra et al., 1999), post-harvest storage (Kim et al., 2003) or growing location (McCracken et al., 2002; George and McCracken, 2003) influence physical and chemical composition of the same wheat cultivar. This makes it difficult to differentiate among them (Table 1). In general "soft-wheat" varieties tend to have higher starch content and higher digestible energy **(DE)** for pigs (Bhatty et al., 1974) and higher starch digestibility and apparent nitrogen-corrected metabolizabe energy **(AMEn)** for broilers (Mollah et al., 1983; Carré et al., 2002; Carré et al., 2003; Skiba et al., 2003) than "hard-wheat" varieties. Contrary, "hard-wheat" varieties have been found to give an improved broiler growth performance compared to "soft-wheat" varieties (Scott et al., 1998; Rose et al., 2001).

GROWING CONDITIONS

The term growing conditions refers to wheat grown at different geographical location, growing season, soil type and rainfall.

Precipitation level mainly influences the carbohydrate composition of wheat. A number of studies have found that drought conditions reduces, grain weight, starch and soluble NSP contents and increases crude protein **(CP)** content, total arabinoxylans, ADF, lignin and free sugar contents of wheat (Brooks et al., 1982; Coles et al., 1997; Ahmadi and Barker, 2001; Kim et al., 2003). Grain composition (specially carbohydrates) is influenced by rainfall during the different stages of wheat growth (vegetative, growing or ripening). Kim et al. (2002) found that total-P content of wheat correlated well with annual precipitation level ($r = 0.478$, $P < 0.05$) but not with precipitation during the growing period. Dusel et al. (1997) reported a positive correlation between annual precipitation and extract viscosity of wheat, while precipitation during the growing season was negatively correlated with extract viscosity of wheat ($r = -0.55$, $P < 0.05$). Rainy conditions just before harvest were related to low specific weight, endosperm hardness and CP content of wheat due to a high proportion of sprouted grains (Metayer et al,. 993; Kruger, 1994; Rose et al,. 2001).

Growing season has an effect on specific weight, endosperm hardness, content of CP and starch and total, insoluble and soluble NSP content of wheat (Metayer et al., 1993; Waldron et al., 1994; Scott et al., 1998; Choct et al., 1999; Rose et al., 2001; George and McCracken, 2003; Kim et al., 2003; Pirgozliev et al., 2003). Nevertheless, most of those effects were mainly explained by differences between environmental conditions nested within the growing season.

Growing location also affects physico-chemical parameters of wheat. In a survey with 7 wheats grown in 10 different locations in France, Metayer et al. (1993) found a between-region effect on

specific weight, thousand grain weight **(TGW)** and starch content. Wheats grown in the North East of France had higher nutritional value compared to the ones grown in Brittany and the South East of France. Longstaff and McNab (1986) found a significant difference between the starch content of two wheat varieties (Norman and Armada) when grown in the North or the South-East United Kingdom. The starch content of wheats was higher when grown in the South-East than in the North United Kingdom. Variability in the CP content of wheat samples has also been correlated with nitrogen application on the growing wheat crops (Uhlen et al., 2004).

If growing conditions affect chemical composition of the wheat, its nutritional value (in terms of energy and animal performance) will be also influenced. Wiseman (1997) reported a 1.1 MJ/kg DM difference (14.6 vs. 15.7 MJ/kg DM) in the DE content of wheat fed to pigs due to growing season. Kim et al. (2004) found that variety, growing season and growing region significantly affected the DE of three different wheat varieties fed to weaner piglets. In that study they concluded that both the variety and the growing conditions, especially precipitation levels during the growing season, were the responsible of the variation observed in DE of wheat (up to 1.9 MJ/kg, on as fed-basis). Mollah et al. (1983) found almost 1 MJ/kg DM difference in AME content caused solely by growing site when fed to broiler chickens. Rose et al. (2001) found that chickens given different wheat batches of the same variety, grown in 1992 harvest year, had significantly lower weight gain and feed intake compared to the 1990 and 1991 harvest year batches. Pirgozliev et al. (2003) reported a growing season effect on AMEn of wheats fed to broilers while animal performance was not affected.

POST-HARVEST STORAGE

Post-harvest storage (period and conditions) alter the chemical composition (Ravindran et al., 2001; Kim et al., 2003) and hence the nutritional value of wheats (Choct and Hughes 1997, 1999; McNab and Knox, 1999; Pirgozliev et al., 2006). Wheat grains harvested and stored at ambient temperature for more than 4 months show a decrease in total starch (**ST**), soluble NSP, acid-detergent fiber (**ADF**) and lignin content and an increase in free sugar content (Jood et al., 1993; Kim et al., 2003). These changes may be the responsible for the observed increase in AME and apparent ileal energy content of stored wheats in broiler chickens diets (Choct and Hughes, 1997, 1999; Ravindran et al., 2001). The activation of various in-seed enzymes and a gradual "in situ" degradation of complex polysaccharides into smaller sugars have been proposed as the mechanism for the changes observed (Choct and Hughes, 1997; Kim et al., 2003). Storage conditions are considered important for the activation of the endogenous phytase in barley and wheat. Ockenden et al. (1997) reported no decrease in phytate content of barley

Table 1. Variation in physical and chemical parameters of some wheat varieties

	Brigadier	Consort	Rialto	Reaper	Source
Hardness					
	79.9[1]	-	85.5[1]	-	b
	56.5[2]	18.0	62.5[2]	55.0[2]	e
HFN[3], s					
	380	-	403	-	b
	425	141	307	214	e
TGW[4], g					
	39.2	39.1	-	43.7	d
	58.3	55.9	54.6	-	a
	46.0	52.8	52.5	57.6	e
SW[5], kg/hl					
	70.5	71.4	-	72.0	d
	77.1	-	76.9	-	b
	77.1	71.6	76.7	70.4	e
Viscosity, cps					
	14.0	6.8	-	14.6	d
	5.6	4.0	9.6	-	a
	2.9	4.0	5.2	6.2	e
	5.2	-	5.7	-	b
Protein, g/kg DM					
	120.0	123.7	-	132.3	d
	108.0	114.4	129.2	-	a
	129.5	85.0	124.0	89.0	e
	145.5	-	151.2	-	b
	-	-	119.0	-	c
Oil, g/kg DM					
	15.4	17.2	-	15.2	d
	17.1	19.5	18.1	-	a
	15.4	18.8	16.1	16.6	e
	14.9	-	16.0	-	b
	-	-	24.0	-	c

Ash, g/kg DM					
	19.5	20.5	-	20.0	d
	15.7	14.9	15.9	-	a
	17.2	11.0	16.9	15.0	e
Starch, g/kg DM					
	626.7	624.7	-	637	d
	680.0	668.0	663.0	724	e
	754.9	-	756.0	-	b
	-	-	664.0	-	c
Insoluble NSP[6]					
	106.2	95.1	-	89.5	d
	75.5	68.0	81.0	66.0	e
	92.8	-	99.6	-	b
	-	-	86.0	-	c
Soluble NSP[6]					
	25.8	19.9	-	22.1	d
	32.0	24.0	35.0	26.0	e
	23.0	-	20.7	-	b
	-	-	29.0	-	c

[1] Measured by NIR analyzer (arbitrary units). [2] Range 0 (soft) to 100 (hard) (relative units). [3] Hagberg falling number. [4] Thousand grain weight. [5] Specific weight. [6] Non-starch polysaccharides.

[a] McNab and Knox, 1999; [b] Rose et al. 2001; [c] Steenfeldt 2001; [d] McCracken et al. 2002; [e] Pirgozliev et al. 2003.

when kept under dry conditions, but a 10% reduction when the samples were kept at higher moisture levels (75% relative humidity). This agrees with Kim et al. (2002), who did not find a reduction of phytate content in wheat samples kept for 6 months at dry conditions. Rehman and Shah (1999) found that wheat grains stored for 6 months at a temperature below 10 ºC did not change their carbohydrate composition while significant biochemical changes were observed when stored at 10, 25 and 45 ºC. The amylase activity of the samples decreased with storage time. Cofie-Agblor et al. (1997) concluded that storage temperatures above 10 ºC increased the heat production of the grain (induced by aerobic and anaerobic respiration). Gras et al. (2000) studied the effect of storage temperature (23, 35, or 40 ºC) and the oxygen concentration (1, 4.6 or 21 %) on the quality of flour milled from stored grain. Results showed that storage temperatures at or below 23 ºC ensured constant flour quality while the oxygen concentration did not affect it. This may indicate that both time and temperature influence the activity of in-seed enzymes.

PHYSICAL FORM OF THE WHEAT GRAIN

Several studies focused on how physical form of the wheat grain fed to broilers affects animal performance, wheat nutrient digestibility and AME (McIntosh et al., 1962; Mollah et al., 1983; Rose et al., 1993; Rose et al., 1995; Rose, 1996; Salah Uddin et al., 1996; Preston et al., 2000; Jones and Taylor, 2001; Svihus and Hetland, 2001; Bennet et al., 2002; Carré et al., 2002; Hetland et al., 2002; Svihus et al., 2002; Carré et al., 2003; Carré, 2004; Svihus et al., 2004; Wu and Ravindran, 2004; Carré et al., 2005). The aim of the present paper is not to review the complexity of the matter but it is important to underline that any physical change of the wheat grain (i.e. steam conditioning, pelleting, grinding, etc.), implies nutrient structure modifications that may affect digestibilities and animal performance. It is known that the addition of whole wheat into broiler diets increases gizzard weight (Preston et al., 2000; Jones and Taylor, 2001; Hetland et al., 2002; Svihus et al., 2002; Wu and Ravindran, 2004), ST digestibility (Svihus and Hetland, 2001; Hetland et al., 2002) and AME (McIntosh et al., 1962; Preston et al., 2000). However, no consistency is reported on broiler performance (McIntosh et al., 1962; Rose et al., 1995; Salah Uddin et al., 1996; Jones and Taylor, 2001; Bennet et al., 2002; Wu and Ravindran, 2004). The reasons for the variation in performance and AME caused by wheat processing are not completely understood and no fixed relationship between processing and the metabolizable energy of cereals for poultry has been established (Burt, 1976; Sibbald 1977; Mollah et al., 1983; Salah Uddin et al., 1996).

CARBOHYDRATES

Carbohydrates constitute up to 80% of the total dry matter of the wheat kernel and variation in their composition (ST vs non ST polysaccharides) has a large effect on the nutritional value of wheat (Table 2). Starch is the predominant polysaccharide (from 59 to 73%), the remaining polysaccharides (cellulose, hemicelluloses, and pentosans) are present in lesser amounts (from 8 to 15%, Table 2 and 3). Soluble carbohydrates are also present in small quantities; monosaccharides (glucose, fructose and galactose); disaccharides (sucrose and maltose), trisaccharides (glucodifructose and raffinose) and other oligosaccharides (glucofructans).

Starch

Starch is composed by two carbohydrate components, both high molecular weight polymers of glucose; amylose and amylopectin. Amylose is an almost linear polymer containing ≈

99% of α-(1-4) and ≈ 1% of α-(1-6) glycosidic linkages. Amylose contains more than 1000 glucose units and it has a molecular weight of around 100 kDa. Amylopectin consists of a heavily branched polymer with ≈ 95% of α-(1-4) and ≈ 5% of α-(1-6) glucose chains. Amylopectin chain ranges from ≈ 12 to 120 anhydroglucose units and its molecular weight is in the order of 104-106 kDa (Morrison, 1993; Buléon et al., 1998; Tester et al., 2004). In most starches amylose represents between 20 to 25% of the ST, although some waxy starches contain very little, if any, amylose (< 1%) while others, high-amylose starches, contain more than 70% amylose (Parker and Ring, 2001; Tester et al., 2004). In wheat, the proportion of amylose ranges from about 18 to 35% (Table 3) although recently waxy wheat mutants have been developed in which amylose represents less than 3% of the ST (Nakamura et al., 1995; Abdel-Aal et al., 2002; Tester et al., 2004).

Native starches contain between 15 and 45% crystallite material (Oates, 1997) in which amylopectin is the responsible of the crystalline structure of the ST granule (Imberty et al., 1991). Three different crystalline forms are known, the A form, typical of cereal starches, consists of ST double helices packed into a monoclinic array. The B form, found in tubers and high amylose cereal starches, is a more highly hydrated and open structure consisting of double helices packed in a hexagonal array (Parker and Ring, 2001). The C form is an intermediate form between A and B (Oates, 1997). The large A granules appear 3 to 7 days after anthesis and increase in size during the grain filling period whereas the B granules are formed 12 to 14 days after anthesis and remain smaller. The C granules are initiated 21 days after anthesis (Bechtel et al., 1990; Parker and Ring, 2001). Wheat A granules have a lenticular shape with diameters of 10-35 μm while B granules are spherical or orthorhombic with diameters of about 2 μm (Parker and Ring, 2001). The A granule starches contain more amylose than the B granule starches (Ando et al., 2002; Ao and Jane, 2007). In wheat, amylose content of the A granules can be 34% vs 27% amylose content of the B granules (Ao and Jane, 2007). The proportion of small and large ST granules, by weight and by number, differed among genotypes (Li et al., 2001) and affect the physico-chemical properties of ST (Ao and Jane, 2007). Hard and soft wheats contain A, B and C starch granules with no obvious differences in morphology and resistance to deformation (Barlow et al., 1973; Bechtel et al., 1993; Turnbull and Rahman, 2002; Brites et al., 2005). However, ST granules from hard and soft wheats differ in the mean surface area (Pitts et al., 1989; Glenn et al., 1992), size-distribution (Bechtel et al., 1993) and shape (Brites et al., 2005).

Starch is the largest component of the mature cereal grain and, in wheat, can comprise as much as 73% of its DM content (Pomeranz and MacMasters, 1968; Carré et al., 2002; McCracken et al., 2002). Most of the AMEn of wheat depends on the utilisation of its ST fraction (content and digestibility) as it is the largest contributor to the energy supply from the grain.

There is a high relationship between ST digestibility and AME values of wheat (Mollah et al.,1983; Rogel et al., 1987b; Wiseman et al., 2000; Wiseman, 2006) but not between ST content and AME (Table 2). Isolated wheat ST has been proved to be almost 100% digestible by chicken pancreatic α-amylase in vitro (Longstaff and McNab, 1986) but large variation (80 to 100%) has been found in situ (Mollah et al., 1983; Rogel et al., 1987b). Chickens secrete enough pancreatic amylase to digest dietary ST completely (Moran, 1982; Longland, 1991). This indicates that other factors within the wheat are responsible for the differences in ST digestibility observed among wheat samples. Characteristics affecting ST digestion are the amylose/amylopectin ratio, proportion of A/B-starch granules, shape and crystallinity of the ST granule, lipid content, nature of the protein matrix surrounding ST granules and the overall architecture of the ST granules. Several studies have shown that the ratio amylose/amylopectin correlates negatively with ST digestion (Åkerberg et al., 1998; Abdel-Aal et al., 2002; StevnebØ et al., 2006) which may be partly explained by the formation of complexes between fatty acids and amylose on the surface of the ST granule (Crowe et al., 2000). The proportion of A-/B- starch granules (large/small granules) is also negatively correlated to ST digestion in wheat and barley (Svihus et al., 2005; StevnebØ et al., 2006; Tester and Karkalas, 2006).

Non-starch Polysaccharides

The term non-starch polysaccharides (NSP) covers a large variety of polysaccharide molecules excluding α-glucans (starch) (Figure 1). Non-starch polysaccharides in cereal grains are predominantly arabinoxylans (pentosans), β-glucans and cellulose. Arabinoxylans content in the wheat grain ranges from 5.68 to 8% DM from which 1.8% DM are soluble (Table 3). The amount of β-glucans is very low (0.8% DM as average) and the amount of insoluble cellulose is 2% DM as average (Table 3).

Arabinoxylans consist of long backbone chains of anhydro-D-xylopyranosyl residues linked together by β-(1→4) glycosidic bonds. Major substituents are single α-L-arabinofuranosyl residues. Also hexoses and hexouronic acids are present sometimes (Fincher, 1975; Amado and Neukom 1985). In addition, phenolics and proteins sometimes have been identified as side chains (Geissmann and Neukom, 1973; Neukom, 1976). Most of the arabinoxylans in cereal grains are insoluble in water but the ones not bound to the cell walls may form viscous solutions due to their capacity to absorb water up to ten times their weight of water (Choct, 1997).

Table 2. Variation in physical (a) and chemical (b) characteristics of wheat and their correlation with AMEn content

a. Physical characteristics

n[1]	Hardness	HFN[2], s	TGW[3], g	SW[4], kg/hl	Viscosity	Correlation with AMEn	Source
12	-	-	-	-	2.07-8.39[5] mPa/s	ns	Austin et al., 1999
22	17-95[6]	-	-	-	1.91-6.03[7] ml/g DM	ns	Carré et al., 2002[8]
15	15-88[6]	-	-	-	2.0-5.6[7] ml/g DM	r =-0.86, P< 0.01 (Viscosity)	Carré et al., 2005[9]
55	-	-	33.5-46.4	59.8-62.1	1.79-5.33[5]	r =0.61, P<0.05 (TGW) r =-0.7, P< 0.05 (Viscosity)	Classen et al., 1995
28	-	-	-	-	0.8-6.5[7] mPas	r=-0.43, P<0.05	Huyghebaert and Schöner, 1999[10]
12	-	-	33.4-47.3	63.2-77.1	5.2-17.5[5]	r =0.85, P<0.05 (SW) r =-0.74, P<0.01 (Viscosity)	McCracken et al., 2002
22	10-29[11]	-	-	-	-	ns	Mollah et al., 1983
25	-	-	27.4-50.0	35.6-40.3	-	ns	Scott, 2005
16	26-83[11]	-	27-45	68-81	0.7-3.2[12] ml/g DM	r=-0.87, P<0.05 (Hardness) r=-0.81, P<0.05 (Viscosity)	Skiba et al., 2003
19	-	-	33-47	-	4.88-81.98[13] cps	ns	Steenfeldt, 2001
20	-	80-436	27.6-50.1	77.0-83.1	0.9-3.1[12]	r=-0.66, P<0.05 (HFN)	Svihus and Gullord, 2002
6	-	88-541	-	57.7-74.2	-	ns	Waldron et al., 1994
50	-	-	34.6-59.3	69.5-80.0	-	ns	Wiseman, 2000

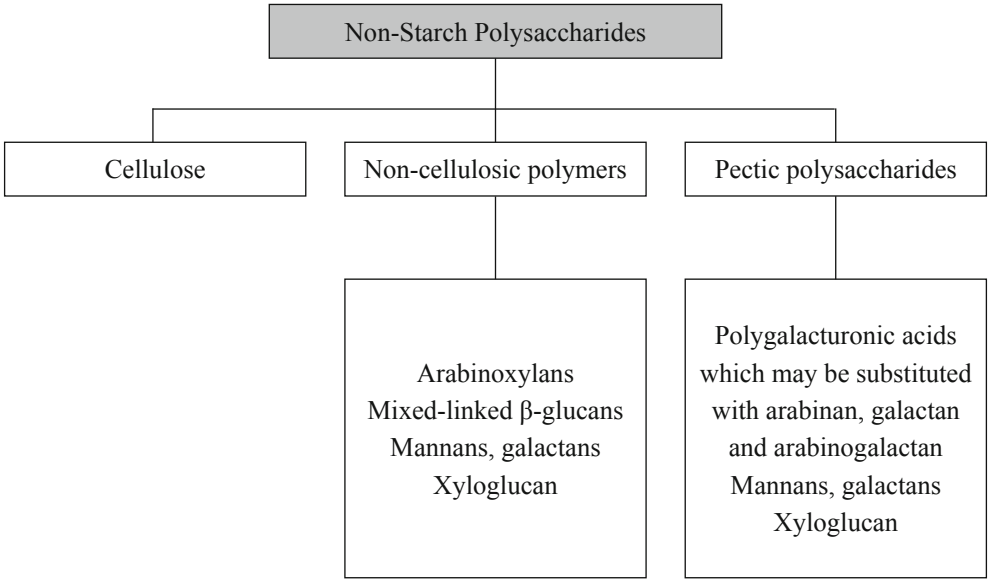
Table 2. Continued

b. Chemical characteristics, g/kg DM

n[1]	Starch	Protein	Soluble NSP	Total NSP	Correlation with AMEn	Source
13	-	-	-	87.6-129.2	r =-0.91, P<0.0001	Annison, 1991
12	-	-	16.8-26.1	91.7-143.5	ns	Austin et al., 1999
22	688-725	96-134	-	-	ns	Carré et al., 2002[8]
15	664-732	107-139	-	-	r =0.58, P< 0.05 (starch)	Carré et al., 2005[9]
81	594-769	89-183	9.3-17.9	81.3-156.8	r =-0.43, P<0.01 (total NSP) r =-0.29, P<0.01 (soluble NSP)	Choct et al., 1999
55	608-647	119-154	18.2-27.9	90.7-107.1	r =-0.7, P<0.05 (soluble NSP)	Classen et al., 1995
28	610-721	132-183	36-101	99-145	r = 0.7, P<0.01 (starch)	Huyghebaert and Schöner, 1999[10]
12	612-656	116-147	15.6-26.3	106-144	ns	McCracken et al., 2002
22	588-719	114-180	-	-	ns	Mollah et al., 1983
25	-	122-199	-	-	ns	Scott, 2005
16	664-733	84-151	-	-	ns	Skiba et al., 2003
16	658-722	112-127	14-39	98-117	r = -0.47, P =0.06 (total NSP)	Steenfeldt, 2001
20	614-712	109-154	-	-	r =0.49, P<0.05 (starch) r =-0.39, P<0.05 (protein)	Svihus and Gullord, 2002
6	650-721	97-129	-	-	ns	Waldron et al., 1994

[1] Number of wheat samples. [2] Hagberg falling number. [3] Thousand grain weight. [4] Specific weight. [5] In vitro viscosity. [6] Measured by NIR analyzer (arbitrary units). [7] Potential applied viscosity. [8] Calculated AMEn (Fisher and McNab, 1987). [9] Calculated AMEn (Carré and Billouet, 1989). [10] Expressed on fresh basis. [11] method of Symes (1961). [12] Water extract viscosity. [13] Viscosity in ileum of diets with two levels of wheat added (650 and 815 g/kg).

Figure 1. Non-starch polysaccharides. Adapted from Bailey (1973)



The NSP fraction of the cereal possesses antinutritive activity even when present at low levels in broiler diets (Annison, 1990; Choct and Annison, 1990, 1992). Inside the NSP, the soluble fraction has been identified as the main responsible for the negative effect as their ingestion increases viscosity of digesta (Bedford et al., 1991; Bedford and Classen, 1992; Schutte et al., 1995), retards the passage of food throughout the intestinal tract (Edwards et al., 1992; Almirall and Esteve-García, 1994), reduces crypts depth and villi density and thickness (Viveros et al., 1994; Yasar and Forbes, 2000), affects the physiology and morphology of the digestive tract (Johnson and Gee, 1981; Angkanaporn et al., 1994) and interacts with the microflora of the gut (Van der Klis and Van Voorst, 1993; Carré et al., 1995). As a consequence, nutrients are not well digested and absorbed and thus cannot be utilised (Choct and Annison, 1990, 1992; Van der Klis et al., 1993a,b).

The insoluble NSP fraction can absorb large amounts of water without changing the motility of the gut (Stephen and Cummings, 1979) and is not degraded by bacterial fermentation in poultry (Choct et al., 1996; Langhout, 1998). Insoluble NSP have been reported to have very little or no effect on nutrient utilisation and AME of the wheat in monogastric animals (Carré, 1990; Annison, 1991; Angkanaporn et al., 1994). However, a number of experiments with poultry have shown a beneficial effect of fermentation in poultry (Choct et al., 1996;

Langhout, 1998). Insoluble NSP have been reported to have very little or no effect on nutrient utilisation and AME of the wheat in monogastric animals (Carré, 1990; Annison, 1991; Angkanaporn et al., 1994). However, a number of experiments with poultry have shown a beneficial effect of moderate levels of insoluble NSP on nutrient digestibility (Rogel et al., 1987a; Hetland and Svihus, 2001; Svihus and Hetland, 2001; Hetland et al., 2003). It has been postulated that an appropriate ratio between the NSP fractions soluble and insoluble may be of importance to reduce the negative effect of the soluble NSP (Choct, 1997). Although the exact mechanism by which insoluble NSP improves nutrient digestibility is not well understood, it seems to relate to its function as digesta passage rate modulator (Hetland et al., 2004). This function is, at least in part, related to particle size as fine grinding has no effect on nutrient digestibility (Rogel, 1985; Hetland et al., 2004).

Total and soluble NSP have been reported to negatively affect AME of wheat (Table 2). Numerous investigators have shown that addition of commercial xylanase enzymes to poultry diets can largely eliminate the adverse effects of NSP in the chick (Petterson and Aman, 1989; Bedford et al., 1991; Bedford and Classen, 1992; Veldman and Vahl, 1994; Bedford, 1995; Schutte et al., 1995; Scott et al., 1998; Steenfeldt et al., 1998a,b; Yasar and Forbes, 2000; Adeola and Bedford, 2004; Choct et al., 2004; Wu and Ravindran, 2004) to the extent that nearly all poultry diets formulated with wheat are supplemented with an arabinoxylanase-based commercial enzyme.

PROTEIN

Protein content of the wheat ranges between 8.7-19% (Table 2 and 3) on a DM basis. It is distributed through all parts of the grain but mostly concentrated in the endosperm (72.5 % of the total protein) and aleurone layer (15.5 % of the total protein). Although the protein content depends mainly on the environment and N fertilisation (Uhlen et al., 2004) the specific composition of the proteins of the endosperm is genetically determined (Wrigley et al. 1982; Carrillo et al. 1988).

Cereal proteins can be classified on the basis of morphology, biological function, solubility or chemical composition (Lásztity, 1984). In wheat it is normally used a classification based on solubility and proposed by Osborne (1907). Hence, albumins are soluble in water; globulins are soluble in salt solutions but insoluble in water; gliadins (also called prolamins) in 70-90% ethanol and glutenins are insoluble in neutral aqueous solutions, saline solutions or alcohol. The former two are classified as cytoplasmic or metabolically active proteins, and the latter two are largely storage proteins. The amount of total storage proteins is highly correlated with CP content of grains with the gliadin fraction correlating better with CP than glutenin (Wieser and

Seilmeier, 1998; Wieser and Kieffer, 2001).

There are important differences in the amino acid composition of cytoplasmic and storage proteins. Storage proteins contain a high proportion of glutamic acid and proline and only a small proportion of lysine, arginine, threonine and tryptophan. Metabolically active proteins contain considerably less glutamic acid and proline, and have higher proportions of lysine and arginine, which give these proteins a higher nutritional value.

Wheat storage proteins (gliadins and glutenins) interact with water to form the gluten complex (rubbery mass containing about 80% of the total protein of the wheat flour). Wheat gluten proteins have been widely studied as they are the only cereal proteins to form a strong, cohesive dough that will retain gas and produce baking products. The bread-making quality of wheat flour is related to the presence and properties of the gluten proteins (Wieser and Kieffer, 2001; Don et al., 2003; Wieser et al., 2006).

Several studies have tried to focus on the relation between the amount of protein in the wheat kernel, and its physical and chemical characteristics, as a way to better define wheat quality. Physically, the most conclusive relationship was found between specific weight (SW) and protein content. This relationship has been reported to be negative by several researchers ($r = -0.62$, $P < 0.05$, $n = 12$; McCracken et al., 2002) ($r = -0.668$, $P < 0.05$, $n = 18$; Kim et al., 2003). Chemically, an inverse relation is thought to exist between protein and ST content (Jenner et al., 1991; Simmonds, 1995; Hucl and Ravindran, 1996; Kim et al., 2003).

Cereal ST granules are embedded in a protein matrix in different degrees (Lasztity, 1984; Classen, 1996). The proteins surrounding the ST granules have to be firstly degraded to expose the ST to amylases and could result in a physical barrier against ST digestion. There is evidence that the protein matrix is a major factor responsible for differences in ruminal digestion of ST (McAllister et al., 1993). However, this has not been demonstrated in broilers, since low ST digestibility would be accompanied by a reduction in protein digestibility and no evidence for this link exists (Wiseman, 2006). The starch-protein interaction (hardness) can also be a factor influencing the ST digestion in broilers. The puroindoline proteins a and b (friabilin protein) are the molecular basis of wheat hardness (Morris, 2002; Hogg et al., 2004). When both puroindolines are present in a wild state, grain texture is soft while it is hard when one of the puroindolines is absent or they have mutations (Morris, 2002). Negative relationships between grain hardness or particle size of wheats and ST digestibility have been reported (Carré et al., 2002; Carré et al., 2003). However, low ST digestibilities have been also observed even when grains have been strongly grinded (Rogel et al., 1987b; Carré et al., 2002).

Table 3. Variation in the different components of starch, protein and NSP of wheat (g/kg DM).

n[1]	Starch	Amylose	Amylopectin	Crude Protein	Glutenin	Gliadin
12	-	-	-	-	-	-
20	615-689	211-335	335-454	-	-	-
5	-	-	-	-	-	-
55	-	-	-	-	-	-
-	-	-	-	-	-	-
28	-	-	-	-	-	-
18	585.2-737.0	193.6-263.4	345.3-543.4	-	-	-
3	-	-	-	103-123	32.2-37.2	55.9-59.4
16	-	-	-	-	-	-
14	-	-	-	87-120[4] 1073-1560[5,6]	291-488[6]	749-1094[6]
24	-	-	-	71.3-145.1[4] 757-1791[5,6]	237-589[6]	495-1202[6]

Soluble fraction shown in parentheses. [1] Number of wheat samples. [2] Arabinoxylans. [3] Expressed on fresh basis.

[4] Protein content of the flour, Nx5.7, fresh basis. [5] Gluten proteins. [6] Absorbance units of HPLC corresponding to 1 mg flour.

No storage influence considered

Total NSP	Arabinose	Xylose	ß-Glucan	Cellulose	Reference
87.6-129.2 (15.2-23.5)	12.4-27.1 (3.1-5.9)	24.1-53.9 (4.0-10.3)	5.6-7.2 (1.2-2.8)	-	f
-	23.5-38.0	33.5-45.7	0.9-8.1	-	b
119 (25)	29 (7)	47 (9)	8	20	d
90.7-107.1 (18.2-27.9)	55.6-68.1[2] (12.0-20.2)		-	-	c
114 (24)	33 (8)	48 (10)	-	20	a
99-145 (36-101)	48-85[2] (10-25)		-	-	g[3]
78.3-110.6 (7.0-14.1)	22.8-35.3 (2.3-4.7)	29.4-44.7 (2.1-5.6)	-	-	j
-	-	-	-	-	k
98-117 (14-39)	22-28 (3-10)	36-47 (3-17)	-	14-21	h
-	-	-	-	-	i
-	-	-	-	-	e

[a] Englyst, 1989. b Annison, 1990. [c] Classen et al., 1995. [d] Bach Knudsen, 1997. [e] Wieser and Seilmeier, 1998. [f] Austin et al., 1999. [g] Huyghebaert and Schöner, 1999. [h] Steenfeldt, 2001. [i] Wieser and Kieffer, 2001.[j] Kim et al., 2003. [k] Konopka et al., 2007.

THE BROILER

The problems associated with the digestion of ST and protein in wheat are considered most important during the first 10 days of age. This is because the gut of the day old chicken is sterile at birth. After hatching and eating, the animal starts to synthesise enzymes (trypsin, chymotrypsin, amylase and lipase) which reach a maximum at day 10 of age (Nitsan et al., 1991; Noy and Sklan, 1995; Sklan and Noy, 2000). During the first 10 days of age there is an increase in the weights of the gastrointestinal tract and an increase in nutrient digestibility (Nitsan et al., 1991) culminating in the ability to produce sufficient and suitable endogenous enzymes to allow the bird to efficiently digest its feed. Digestion of the fiber fraction of the wheat by the birds own processes is poor, as it lacks the capacity to synthesize suitable enzymes (particularly arabinoxylanases). It relies on a resident microbial flora in its caeca to achieve fiber fermentation, and release of nutrient from that fraction of the diet.

Accessibility of the substrate by various enzymes (both endogenous and microbial) can affect total wheat digestion. The ST granules of the endosperm are embedded in a protein matrix. Processing of the wheat (milling) and/or the grinding action of the gizzard disrupt the starchy endosperm of the wheat and increase the surface area allowing better binding of the amylases. Wheat ST and wheat gluten can be almost 100% digestible and hence, their nutritive value for the animal can also be considerable. The rate of digestion of the different wheat proteins has not been reported yet. However, it seems to be very high since the ST granules are embedded in the protein matrix and ST has been reported to be rapidly digested (Waldron et al., 1995; Wiseman et al., 2000; Weurding, 2002). Nevertheless, the difference in the nutritive value of wheats may come from the combination of the digestion rate of the protein first and of the ST subsequently after. Waldron et al. (1995) found significant differences in rate of starch hydrolysis between two wheat varieties (Dean and Beaver) with Dean samples showing 29% greater rate of ST hydrolysis than that in Beaver samples. It appears that ST digestion rate may partly explain differences in animal performance (Waldron et al., 1995; Wiseman et al., 2000; Weurding, 2002).

It is well documented that modern broilers are unable to adjust feed intake of diets to achieve a required nutrient intake to support growth (Forbes, 2005; Scott, 2007). Recent research suggested that limitations in feed intake of broiler chickens are the main responsible of the low growth and the high fedd conversion ratio observed (Scott et al., 1998; Scott, 2004; Scott, 2007). The same authors postulated that what most affects voluntary feed intake is digesta passage rate, particularly in wheat-based diets, and that limitati-

ons in passage rate could be due to variability in diet hydratation time. Other factors affecting digesta passage rate are composition of the feed, i.e. polysaccharides content (Van der Klis and Van Voorst, 1993), NSP-degrading enzymes (Almirall and Esteve-García, 1994), fat sources (Dänicke et al., 1997), feed form (Hetland and Svihus, 2001) and raw material particle size (Carré, 2000; Svihus et al., 2002).

CONCLUSIONS

Based on the literature presented it seems that the nutritive value of the wheat grain cannot be clearly predicted from the traits measured so far. Intrinsic and extrinsic factors have been widely studied, along with certain digestibility characteristics, and it is clear that these may influence both wheat AME value and animal performance. Currently it is not possible to quantify AMEn value of the wheat from calculations involving the actual studied properties of the wheat as reported in animal studies. Moreover, the relationship between wheat AMEn value and animal performance is not constant, as two wheats with the same chemical composition may deliver different AMEn values. Equally, two wheats with the same AMEn value may give different animal performance, or vice versa. It is clear that there are other, as yet unidentified, factors within wheat that affect wheat AMEn and animal performance. Botanically, genetic variety influences the nutritive value of wheat, mainly via its chemical composition. However, wheat breeding programs continuously generate modifications to the same variety. This makes it impossible to use variety as a trait to determine nutrient composition and digestibility properties. The type of wheat and its growing conditions are more important for predicting its nutritional feeding value than other traits. Chemically, the wheat grain can be considered one of the most intensively studied cereals. Starch, the largest component of wheat, is the major contributor to the AMEn of wheat. However, all the attempts to relate ST content or ST components with wheat AMEn have been inconsistent. Still, ST digestibility and ST digestion rate seem to be important traits related to wheat AMEn and animal performance.

Non-starch polysaccharides are anti-nutritional factors in non-ruminants because the animals do not synthesise the enzymes to break them down. The solube NSP fraction of wheat has been identified as being responsible of the high intestinal viscosity in broiler chickens and has been put forward as one of the main factors influencing negatively wheat AMEn and poultry performance. The use of commercial NSP exogenous enzymes is well documented, and is known to reduce the intestinal viscosity, having a positive effect on animal performance.

The protein fraction, mainly located in the endosperm, represents up to 16% of the wheat grain and surrounds the ST granules as a matrix. Although it dictates some of the dough properties of wheat, and is important in the bakery industry, its influence in animal nutrition has not been as widely studied. Although wheat ST and gluten may be highly digestible, the difference in the nutritive value of wheat may come from the combination of the digestion rate of the protein first and of the ST subsequently. Changes in growing conditions of the wheat crop, can affect both physical and chemical parameters of the wheat grain, as well as harvest yield. Protein and ST digestion are also thought to vary between wheats grain.

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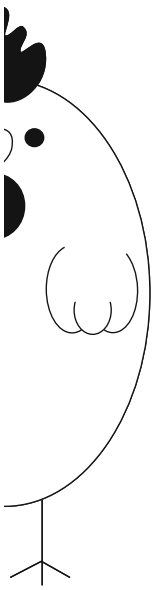
CHAPTER 3

Physico-Chemical Characteristics of Wheat and Their Influence on Starch Digestion and Wheat Apparent Metabolizable Energy in Broiler Chickens



ABSTRACT A series of studies were conducted to determine the effect of different physico-chemical properties of 50 wheat samples on starch **(ST)** digestibility and on wheat nitrogen-corrected apparent metabolizable energy **(AMEn)** in broiler chickens by using Pearson correlations and stepwise regression analysis. Wheats were collected from five different European countries (France, Ireland, Portugal, Spain and The Netherlands) to cover the range of wheats used in European feed formulation for broiler chickens. Wheats were included into nutritional complete mash diets. The diets contained high amounts of the test wheats, 55% (for 18 wheats) or 70% (for 32 wheats). In all experimental diets starch from wheat was the only ST source. Each diet was offered ad libitum to 192 broiler chickens from d 21 to d 30 and ST digestibility and wheat AMEn were determined. Chromic oxide (Cr_2O_3) at an inclusion rate of 0.5% in the diet was used as indigestible marker. Wheat crude protein **(CP)** and ST content were negatively related ($r = -0.742$, $P < 0.0001$). Starch digestibility was similar at the ileal and fecal level (average 95%, range from 89.67 to 93.99%). Wheat AMEn varied from 2,865 to 3,589 kcal/kg dry matter **(DM)** (average 3,262 kcal/kg DM). Only ST content of wheat positively influenced ileal and fecal ST digestibility ($r = 0.469$, $P = 0.002$). Crude protein content in wheat had a negative effect on wheat ST digestibility at both ileal and fecal level ($r = -0.567$, $P = 0.0001$). Compared to CP a lower but significant relationship was observed between NDF content and ST digestibility ($r = -0.372$, $P = 0.017$). The equations on parameters relating AMEn with physico-chemical properties of wheat samples (as currently measured via near-infrared spectroscopy **(NIRS)** in the feed mills) did not show a good prediction of wheat AMEn (R^2 from 0.17 to 0.44, RSD = 142 and 120 kcal/kg DM, respectively). Stepwise regression analysis showed that ST (digestible starch) is a consistent factor for predicting wheat AMEn. However, the equations to predict AMEn with the use of ST only or by the more appropriate trait of digestible ST showed a similar determination coefficient (0.17 and 0.18, respectively).

(Key words: broiler, wheat, chemical composition, physical parameters, metabolizable energy)

INTRODUCTION

Wheat grain is the main cereal used in European broiler diets with inclusion percentages up to 55-60% contributing approximately to 65% of the dietary metabolizable energy. Under these circumstances, the utilization of wheat in broiler formulation requires an adequate knowledge of its nutritive value. If inadequate determination of nutritional value is made, the feed offered for broilers can have an incorrect energy to protein ratio. As a consequence, broiler chickens cannot show their genetically determined potential growth and therefore important economic losses in this type of production and in the feed industry will occur.

The nutritive value of wheat is mostly defined by its energy value (nitrogen-corrected apparent metabolizabe energy, **AMEn**) and amino acids digestibility. The AME content of a raw material depends mostly on its chemical composition and on the availability of its nutrients to the animal. Research through years have tried to identify the factors that influence wheat AMEn in an attempt to obtain an adequate prediction equation for wheat AMEn. However, despite the efforts, no very high correlation between AMEn and phsysico-chemical properties in wheat has been established (Wiseman 2000; Steendfeldt, 2001). For example, Classen et al. (1995) found a negative relationship between viscosity and wheat AMEn ($r = -0.7$, $P < 0.05$), whereas Austin et al. (1999) failed to find such a correlation. Huyghebaert and Schöner (1999) positively correlated starch **(ST)** content to AMEn ($r = 0.70$, $P < 0.01$) but this was not supported by Skiba et al. (2003).

Starch is the major energy yielding component of wheat. Therefore, any variability in its content or digestibility would influence the AMEn value of wheat. Although it is generally assumed that ST is well digested by broiler chickens (Longstaff and MacNab, 1986), some research papers have shown wheat ST digestibility to range from 80 to 99% when fed in situ to broiler chickens (Mollah et al., 1983; Rogel et al., 1987). Rogel at al. (1987) reported that ST isolated from wheats with low AMEn were 100% digested by broiler chickens. Therefore, it seems that the observed variability in ST digestibility is linked to other factors different that ST per se.

Additionally, most previous studies on wheat have been confined to only one or two physico-chemical parameters (ie, ST, non-starch polysaccharides **(NSP)**, hardness or particle size, specific weight (**SW**)) (Choct and Annison, 1990; McCracken and Quintin, 2000; Svihus and Hetland, 2001). Therefore, wheat samples are chosen to obtain a large range in these parameters with any other nutrient variability unknown. Moreover, the information given in the research papers is often very limited in regard to the nutrient composition of the wheat samples chosen. However, wheat grains received at the feed mill are normally a mixture of different cultivars which are not classified by a specific nutrient content. In addition, the quality

control of wheats after arrival at the feed mill implies only the elimination of contaminated or dirty samples with no study on wheat chemical properties as a reason for "yes rejection" or "no rejection" of the sample.
The objective of the present study was therefore to analyze the effect of physico-chemical characteristics of 50 wheat samples received at different feed mills around Europe on the nutritive value of wheat for broiler chickens with special attention on starch.

MATERIAL AND METHODS

Wheats and Feeds

A total of 50 wheat samples were obtained from different feed mills in France, Ireland, Portugal, Spain and The Netherlands without specific information on cultivar or growing conditions. Wheat samples were incorporated at a level of 55% (for 18 wheats) or of 70% (for 32 wheats) to formulate the diets. The diets met or exceeded the nutrient requirements of broiler chickens (NRC, 1994). The range of the ingredient composition and the determined analysis of the experimental diets used in the different experiments is shown in Table 1. In all experimental diets, starch from wheat was the only ST source. Chromic oxide ($\mathbf{Cr_2O_3}$) at 0.5% was used as indigestible marker. Wheats were milled separately and passed through a 2.5 mm screen in a hammer mill (Rosal, 40 horse power, Barcelona, Spain) and then mixed with the other ingredients to constitute the experimental diets. Diets were offered as mash.

Animals and Experimental Design

Several trials were conducted under the same conditions during a period of 2 consecutive years. For each trial, 400 day-old male Ross-308 chickens were obtained from a commercial hatchery (Cazalegas, Toledo, Spain). From hatching until 21 d of age, chickens were randomly assigned to 4 pens (7.5 m^2) of 100 chickens each and fed on a mash standard diet covering all nutrient requirements (NRC, 1994). Light was on continuously during the first day and during 16 hours per day afterwards. Broiler chickens were kept at 32ºC during the first wk of age. Thereafter temperatures decreased with 3ºC per wk to reach 22ºC at 21 d of age. At d 21, chickens were individually weighed, 192 of them were selected for the experiment (based on same body weight) and moved to another room with metabolic cages (19.5 x 38.5 x 35 cm, width x length x height). Animals were randomly assigned to the dietary treatments (different wheats) and individually allocated to the metabolic cages until 16 replicates per treatment were obtained. The cages were located in a room with 16 h light/day and ambient temperature around 22ºC. Feed and water were provided ad libitum during the whole trial.

Table 1. Range of the ingredient profile of the experimental diets used in the different experiments

	Experimental diets	
Ingredient, %		
Wheat	70.00	55.00
Soya full fat	-	27.00
Soybean meal	23.13-25.48	10.41-11.34
Soybean oil	0.75-2.00	2.38-3.90
Salt	0.01-0.25	0.23-0.30
Limestone	1.27-1.63	1.22-2.00
Monocalcium phosphate	0.95-1.32	1.10-1.14
Sodium bicarbonate	0.20-0.43	0.18-0.28
L-lysine	0.02-0.45	0.18
L-threonine	0.05-0.21	0.06
DL-methionine	0.09-0.46	0.22-0.29
Mineral-vitamin premix	0.30[1]	0.30[1]
Determined analysis, %		
Dry matter	90.01-91.25	87.85-91-50
Crude protein	19.00-23.06	22.24-25.24
Ether extract	2.20-6.20	8.16-15.26
Ash	4.96-6.68	6.92-7.72
Starch	40.70-44.35	36.38-41.87
Crude fiber	2.61-3.76	2.95-4.32
Gross energy (kcal/kg DM)	3,883-4,071	4,505-4,735

[1] Supplied per kg of diet: cobalt , 0.2 mg; copper, 8 mg; iron, 20 mg; manganese, 80 mg; zinc, 59 mg; selenium, 0.2 mg; iodine, 2 mg; vitamin A, 9,999 IU; vitamin E, 29 IU; cholecalciferol, 50 µg; vitamin B_1, 2 mg; vitamin B_2, 8 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 0.01 mg; vitamin K, 3 mg; pantothenate, 10 mg; folic acid, 1 mg; niacin, 50 mg; biotin, 0.1 mg; B.H.A plus ethoxyquine, 100 mg; salynomicine 70 mg.

The experimental period lasted 9 d (from d 21 to d 30) for each trial. Excreta was collected daily for 3 consecutive days, from d 27 to d 30. Contaminations, such as down and feathers, were carefully removed and the excreta was stored in containers at –20ºC. Afterwards, excreta was dried at 70 ± 0.5ºC during 48 hours. Samples collected from each bird during the 3 d of excreta collection were blended, ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored in plastic tins until analysis. Samples were analyzed for ST, nitrogen (**N**) and gross energy **(GE)** to determine ST digestibility, N retention and AME. The AME was corrected to zero N balance according to Hill and Anderson (1958).
At the end of the experiment (d 30), from six out of seven trials, all birds (n = 40) were euthanasied following the principles for care of animals in experimentation (Spanish Royal Decree 1201/2005, 2005). Immediately afterwards the small intestine was removed. The ileum, defined as the area between the Meckel's diverticulum and the ileocaecal junction was dissected and the digesta from this area was collected by gentle finger-stripping and the digesta was freeze-dried. Ileal contents of two animals from the same treatment were pooled. So in total, 8 replicates from each treatment diet were used for statistical analysis. Samples were ground through a 0.75 mm sieve and stored in plastic bags until analysis. Ileal samples were analyzed for ST, N and chromium.

Chemical Analysis

All analysis, except Cr_2O_3 in feed (9 times/treatment), were carried out in duplicate and results are reported on dry matter **(DM)** basis. Chemical analysis of the wheats, diets and excreta were conducted according to the methods of AOAC International (1995) for DM (930.15), N (954.01), crude fiber **(CF)** (962.09), ether extract **(EE)** (960.39) and ash (942.05). Neutral-detergent fiber **(NDF)**, acid-detergent fiber **(ADF)** and acid-detergent lignin (ADL) were determined following the procedures described by Van Soest et al. (1991). Starch content in wheat samples and experimental diets were analyzed polarimetrically (Table 1 and 2, Spanish Royal Decree 2257/1994, 1994). The alpha-amylogluclosidase method (996.11, AOAC International) was used to determine the ST content of ileal digesta, excreta and experimental diets to calculate ST digestibility. Chromium oxide content in feed and excreta was determined according to Fenton and Fenton (1979). *In vitro* viscosity of wheat samples was measured according to Bedford and Classen (1993). Gross energy values were determined by bomb calorimetry using a Parr 6100 adiabatic calorimeter (Parr Instrument Company, Moline, IL, USA).

Calculations and Statistical Analysis

The following equations were used for calculation of apparent total tract digestibility, apparent ileal digestibility and AMEn content of experimental diets:

$$\text{Apparent total tract ST digestibility (\%)} = \{1 - [(Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ excreta}) \times (ST_{\%\ excreta} / ST_{\%\ diet})]\} \times 100,$$

$$\text{Apparent ileal ST digestibility (\%)} = \{1 - [(Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ digesta}) \times (ST_{\%\ digesta} / ST_{\%\ diet})]\} \times 100,$$

$$\text{AMEn (kcal/kg of diet)} = GE_{kcal/kg\ diet} - [GE_{kcal/kg\ excreta} \times (Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ excreta})] - 8.22 \times \{N_{\%\ diet} - [N_{\%\ excreta} \times (Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ excreta})]\}$$

where GE is gross energy, Cr_2O_3 is chromic oxide, ST is starch, N is nitrogen and 8.22 is the energy equivalent of 1 g of uric acid N.

The AMEn values of the different wheat samples were calculated following the methodology described by Villamide (1996). For that purpose, in each trial three other diets were formulated with increasing concentrations (60, 70 and 80%) of a reference wheat, replacing a high protein basal diet. The AMEn of the reference wheat was calculated by extrapolation to 100% of inclusion of the linear regression equation between dietary AMEn and wheat level. The AMEn value of wheat samples were calculated by difference to the AMEn value of the reference wheat.

Statistical analysis was performed using the SAS software (SAS Institute, 1985). Correlation coefficients among all physico-chemical properties and nutritional value of the wheat samples were performed using a simple Pearson correlation analysis. Relationships were examined using the mean values of the diets instead of the individual bird responses. Stepwise analysis was performed to find a prediction equation to estimate wheat AMEn value from physico-chemical and digestibility characteristics of the different wheat samples. All statements of significance were based on a P-value of equal to or less than 0.05.

RESULTS

The influence of feed formulation (wheats included at 55 or 70%) and trial effect on ST, dietary crude protein (**CP**) digestibility and wheat AMEn were primarily studied. Dietary CP digestibility was influenced by the level of inclusion of wheat in the feed formulation ($P < 0.001$) whereas ST digestibility and wheat AMEn were not significantly affected ($P = 0.50$ and 0.92, respectively). Therefore, the results obtained in all the assays were pooled to analize ST digestibility and wheat AMEn (Table 2), whereas two groups were done to analyze the dietary CP digestibility (Table 3).

Table 2. The mean, standard deviation and range (minimum vs maximum) of physico-chemical parameters (n = 50), starch digestibility (n = 40) and AMEn (n = 50) of wheat samples

Item[1]	Mean	Std Dev	Minimum	Maximum
Physico-chemical parameters, % DM				
Dry matter	88.23	1.96	84.59	92.70
Crude protein	13.31	2.35	8.79	19.02
Ash	1.77	0.19	1.50	2.25
Starch	66.16	2.52	59.58	70.82
Ether extract	1.68	0.21	1.26	2.37
Crude fiber	2.65	0.22	2.16	3.21
NDF	16.80	2.14	12.95	21.31
ADF	3.50	0.45	2.52	4.45
ADL	0.97	0.18	0.57	1.33
Gross energy, kcal/kg DM	4,396	42.57	4,312	4,484
Specific weight, kg/hl	75.76	2.93	67.50	82.90
Viscosity, cp	5.37	1.64	3.13	10.20
Digestibility (%) and AMEn (kcal/kg DM)				
Ileal ST digestibility	94.18	2.34	89.30	98.69
Fecal ST digestibility	95.80	2.21	90.05	98.97
AMEn	3,262	155.10	2,865	3,589

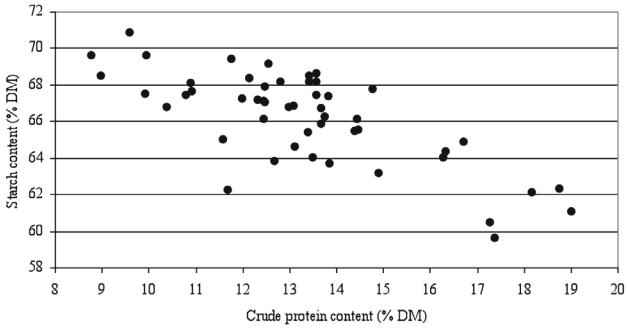
[1] *NDF = neutral-detergent fiber; ADF = acid-detergent fiber; ADL = acid-detergent lignin; AMEn = nitrogen-corrected apparent metabolizable energy.*

Wheat samples used in the present study had a wide variation in physico-chemical characteristics, mainly in viscosity (from 3.13 to 10.20 cp, 30% CV) and CP content (from 8.79 to 19.02%, 18% CV) (Table 2). The nutrients with the lowest variation were ST (CV = 3.8%, range from 59.58 to 70.82%) and SW (CV = 3.9%, range from 67.50 to 82.90 kg/hl). The majority of the samples (84%) had a CP content (on DM basis) below 15% with 5 samples showing a very low CP content (< 10%) and 8 samples showing a CP content greater than 16% (Figure 1a). Most samples (52%) had a ST content from 66 to 68.50% (on DM basis). Total wheat ST digestibility was high (average 95.80%, 2.30% CV) and ranged from 90.05 to 98.97% (Table 2). Similar ST digestibility values at ileal and fecal level were observed

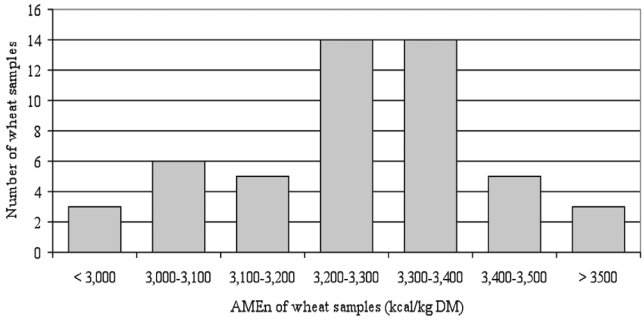
Table 3. The mean, standard deviation and range (minimum vs maximum) of dietary crude protein (CP) supplied by the wheat and ileal crude protein digestibility of diets when wheat was included at 55% (n = 18) and 70% (n = 21)

Item, %	Mean	Std Dev	Minimum	Maximum
Wheats included at 55%				
Dietary CP supplied by the wheat	28.30	3.63	21.30	33.93
Dietary ileal CP digestibility	81.20	1.31	78.81	83.14
Wheats included at 70%				
Dietary CP supplied by the wheat	41.66	5.51	31.11	50.81
Dietary ileal CP digestibility	74.64	2.62	69.95	79.34

Figure 1. Distribution of the wheat samples (n = 50) used in the current work.
a. Relationship between crude protein and starch content



b. Histogram of the AMEn of the wheat samples



for each wheat sample. Wheat AMEn showed a wide range of variation (from 2,865 to 3,589, CV = 5%) (Table 2) although most samples ranged from 3,200 to 3,400 kcal/kg DM (Figure 1b).

The inclusion of wheat samples at 55 or 70% implied different dietary CP content supplied by the wheat (Table 3). Wheats included at 55% supplied as average 28.30% (on DM basis) of the dietary CP content whereas wheats included at 70% supplied as average 41.66%. Ileal dietary CP digestibility ranged between 78.81 to 83.14% and between 69.95 and 79.34% for diets containing 55% and 70% of wheat, respectively.

Table 4. Correlation matrix for physico-chemical characteristics of wheat samples (n = 50). Significance between brackets.

Item[1]	CP	ASH	ST	EE	CF	NDF	ADF	ADL	SW	VISC
CP	1	0.160 (0.266)	-0.742 (<0.0001)	0.193 (0.178)	-0.126 (0.380)	0.168 (0.241)	-0.001 (0.993)	0.188 (0.189)	-0.096 (0.506)	-0.017 (0.902)
ASH		1	-0.200 (0.163)	0.288 (0.042)	0.272 (0.055)	-0.039 (0.786)	0.170 (0.237)	0.255 (0.072)	-0.285 (0.044)	-0.340 (0.015)
ST			1	-0.384 (0.006)	0.024 (0.867)	-0.144 (0.317)	-0.104 (0.469)	-0.183 (0.202)	0.284 (0.045)	-0.006 (0.966)
EE				1	0.066 (0.645)	0.128 (0.374)	0.272 (0.055)	0.111 (0.440)	-0.374 (0.007)	-0.294 (0.038)
CF					1	0.116 (0.420)	0.506 (0.0002)	0.083 (0.563)	-0.261 (0.066)	-0.084 (0.561)
NDF						1	0.015 (0.912)	-0.094 (0.515)	-0.287 (0.042)	-0.266 (0.061)
ADF							1	0.409 (0.003)	-0.164 (0.252)	-0.023 (0.871)
ADL								1	-0.279 (0.049)	-0.188 (0.189)
SW									1	0.092 (0.522)
VISC										1

[1] CP = crude protein; ST = starch; EE = ether extract; CF = crude fiber; NDF = neutral-detergent fiber; ADF = acid-detergent fiber; ADL = acid-detergent fiber; SW = specific weight; VIS = viscosity.

Starch content, the main component of the wheat grain, negatively correlated with CP (r = -0.74, P < 0.0001) and with EE (r = -0.384, P = 0.006) and positively with SW (r = 0.28, P = 0.045) (Table 4). Wheat EE negatively correlated with both SW and viscosity (r = -0.37 and -0.29, P < 0.05, respectively). The fiber fractions NDF and ADL negatively correlated with SW (r = -0.29 and -0.28, P < 0.05).

Table 5. Correlation coefficients between physico-chemical characteristics of wheat samples and the ileal and fecal starch digestibility (n = 40) and wheat AMEn (n = 50) in broiler chickens. Significance between brackets.

Item[1]	Ileal starch digestibility	Fecal starch digestibility	Wheat AMEn
CP	-0.609 (<0.0001)	-0.567 (0.0001)	-0.280 (0.029)
ASH	-0.023 (0.883)	-0.062 (0.702)	-0.170 (0.235)
ST	0.447 (0.003)	0.469 (0.002)	0.418 (0.002)
EE	-0.388 (0.031)	-0.287 (0.072)	0.060 (0.677)
CF	0.340 (0.031)	0.180 (0.265)	0.132 (0.360)
NDF	-0.419 (0.007)	-0.372 (0.017)	0.240 (0.092)
ADF	0.097 (0.551)	0.202 (0.211)	-0.072 (0.616)
ADL	-0.185 (0.250)	0.139 (0.391)	-0.301 (0.033)
SW	0.228 (0.156)	0.087 (0.590)	0.070 (0.624)
VISC	0.236 (0.141)	0.098 (0.545)	-0.136 (0.344)

[1] CP = crude protein; ST = starch; EE = ether extract; CF = crude fiber; NDF = neutral-detergent fiber; ADF = acid-detergent fiber; ADL = acid-detergent lignin; SW = specific weight; VISC = viscosity.

Correlation coefficients between physico-chemical characteristics and nutritive value of wheat samples are shown in Table 5. The only parameter that positively correlated with ST digestibility was the ST content ($r = 0.44$, $P = 0.003$ and $r = 0.47$, $P = 0.002$ for ileal and fecal ST digestibility, respectively) whereas the other parameters correlated it negatively or did not correlate. Thus, wheat CP negatively affected ileal ($r = -0.61$, $P < 0.0001$) and fecal ST digestibility ($r = -0.57$, $P < 0.0001$). The same observation was shown for NDF ($r = -0.42$, $P = 0.007$ and $r = -0.37$, $P = 0.017$ for ileal and fecal ST digestibility, respectively) and for EE ($r = -0.38$, $P = 0.031$, for ileal ST digestibility). In the same way, wheat AMEn was positively correlated with ST ($r = 0.42$, $P = 0.002$) but negatively with CP ($r = -0.28$, $P = 0.029$) and with ADL ($r = -0.30$, $P = 0.033$).

Several different prediction equations for wheat AMEn based on stepwise analysis were run and results are given in Table 6. When the parameters commonly determined in the feed mills (via NIRS) were offered (DM, ST, CP, EE and CF) the stepwise only included ST (equation 1), explaining 17% of the variation in wheat AMEn. When fiber was expressed as NDF, ADF and ADL, stepwise analysis included NDF (equation 2) as a positive term. The determination coefficient increased to 0.27 and the RSD was slightly reduced to 135 kcal/kg DM. When all physico-chemical parameters were offered in the statistical analysis CP and GE entered in the equation with a negative and positive coefficient, respectively. The determination coefficient increased to 0.44 and RSD was reduced to 120 kcal/kg DM.

If wheat AMEn was predicted only by digestible ST (ileal or fecal digestible ST) (equation 4, $n = 40$) the term included was fecal digestible ST. The determination coefficient (0.18) was similar to equation 1 ($n = 50$) and the RSD was 142 kcal/kg DM (equation 4).

The best prediction equation of wheat AMEn was obtained by combining physico-chemical and digestible properties of wheats (equation 7). The equation included EE, NDF, GE and fecal digestible ST as positive terms and ADL as the only negative term ($R^2 = 0.58$, RSD = 118 kcal/kg DM).

DISCUSSION

This study was designed to determine the possibility of predicting wheat AMEn from the different physico-chemical and digestibility properties of the wheat grains commonly received at European feed mills. For this reason, wheat samples were not selected by any parameter except the proper quality control of the fed mill (i.e. no dust, no contamination, etc.).

It is known that physico-chemical properties of the wheat grains are greatly influenced by location at which they are grown (including growing and environmental conditions) (Choct et al., 1999; Kim et al., 2003). The fact that the wheat samples were collected from 5 different European countries (France, Ireland, Portugal, Spain and The Netherlands) increased the variability in their physico-chemical properties (mainly CP, ADL and viscosity). So the vari-

ability in these samples is higher compared to other studies (Svihus and Gullord, 2002; Carré et al., 2005). The wide range of variation observed in CP content (from 8.79 to 19.02%) was probably due to N fertilization practices (Uhlen et al., 2004), whereas the high variation observed in viscosity and ADL could be explained by the influence of different climatic conditions (Choct et al., 1999; Kim et al., 2003). The relatively low range in wheat ST content may be explained by the fact that feed mills usually ask for wheat grains with high SW (above 72 kg/hl), which positive correlates with ST content (Table 4).

Most of the European feed mills use a NIR equipment to derive prediction equations for AMEn from values of different chemical parameters mainly DM, ST, CP, EE and CF. When those parameters were offered to the stepwise analysis only ST was chosen, according to the positive correlation between ST content and wheat AMEn showed in this experiment ($r = 0.418$, $P = 0.002$) and reported by others (Carré et al., 2005). This is logical since ST is the main energy component of the wheat grain (Longstaff and McNab, 1986). The equation showed a RSD of 142 kcal/kg DM which indicates a high degree of uncertainty when ST is used as the only parameter to predict wheat AMEn. When the fiber classification of Van Soest et al. (1991) was used, the variable NDF was added to the equation and the accuracy of the prediction increased (RSD = 135 kcal/kg DM). Additionally, the best prediction equation using only physico-chemical properties of the wheat introduced CP, ST, NDF and GE ($R^2 = 0.44$, RSD = 120 kcal/kg DM). However, neither NDF nor GE are parameters commonly measured in wheat grains and their determination is time consuming. Therefore, to develop a NIR equation to predict NDF and GE when the maximum determination coefficient obtained is 0.44 and the RSD is equal to 120 kcal/kg DM seems to be inadequate.

A more appropriate way of predicting wheat AMEn (when based on ST) would be by the use of digestible ST (Longstaff and McNab, 1986). The digestibility of the ST in the current study was high (average 95%) which agrees with the general assumption that ST is well digested by broiler chickens (Annison, 1990; Carré, 2004). However, in the present study certain variability in ST digestibility (from 90 to 98%) was also observed. Moreover, ST digestibility at ileal and fecal level were similar indicating no further ST digestion in the hind gut as discussed by Weurding et al. (2001). Surprisingly, the introduction of the term digestible ST instead of ST alone did not increase the accuracy of the prediction (RSD = 156 kcal/kg DM) which could be explained by a lower number of samples used ($n = 50$ vs $n = 40$ for prediction based on ST content or on digestible ST, respectively). This agrees with what observed by other authors (Mollah et al., 1983; Rogel et al., 1987). Rogel et al. (1987) reported that those ST samples isolated from wheats which show in situ a low ST digestibility (< 90%) were digested completely by

Table 6. Prediction equations (stepwise) for wheat AMEn from chemical analysis (n = 50) and digestible properties (n = 40) of wheat

Parameters offered % DM	(Equation) AMEn prediction equation[1,2] kcal/kg DM	R^2	RSD	P
	Based on physico-chemical wheat characteristics (n = 50)			
DM, ST, CP, EE, CF	(1) AMEn = 1,556 + 25.78 ST	0.17	142	0.0025
DM, ST, CP, EE, NDF, ADF, ADL	(2) AMEn = 1,001 + 28.51 ST + 22.22 NDF	0.27	135	0.0007
All	(3) AMEn = -8,517 + 32.22 ST + 23.35 NDF – 29.47 CP + 2.19 GE	0.44	120	<0.0001
	Based on digestibility properties of wheat characteristics (n = 40)			
Dig ST (il), Dig ST (fe)	(4) AMEn = 1,941 + 20.7 Dig ST (fe)	0.18	156	0.0058
	Based on physico-chemical and digestibility properties of wheat (n = 40)			
DM, ST, CP, EE, CF, Dig ST (il), Dig ST (fe)	(5) AMEn = 808.14 + 371.41 EE + 28.47 Dig ST (fe)	0.32	144	0.0007
DM, ST, CP, EE, NDF, ADF, ADL, Dig ST (il), Dig ST (fe)	(6) AMEn = 630.07 + 339.41 EE + 20.80 NDF – 240.68 ADL + 30.39 Dig ST (fe)	0.48	129	<0.0001
All	(7) AMEn = -6,601 + 291.66 EE + 23.95 NDF – 260.38 ADL + 1.48 GE + 42.06 Dig ST (fe)	0.58	118	<0.0001

[1] CP = crude protein; ST = starch; EE = ether extract; CF = crude fiber; NDF = neutral-detergent fiber; ADF = acid-detergent fiber; GE = gross energy (kcal/kg DM); Dig ST (il) = ileal digestible ST; Dig ST (fe) = fecal digestible ST.

[2] All variables left in the model are significant at $P < 0.05$.

6 wk broiler chickens. This suggests that other factors that ST per se are responsible for variations in ST digestibility.

Looking into the correlation matrices (Tables 4 and 5), a strong negative relationship between CP and ST content and ST digestibility was found ($r = -0.742$ and -0.567, $P < 0.001$, ST content and fecal ST digestibility, respectively). The negative relationship between CP and ST has been reported previously (Hucl and Ravindran, 1996; Kim et al., 2003). This finding can be explained by physical filling of the wheat grain together with the grain yield (Hucl and Ravindran, 1996). Moreover, the endosperm of the wheat grains is mainly formed by ST granules embedded in a protein matrix (Hoseney, 1998) which may give a physical barrier against digestion.

We tried to estimate the CP digestibility of wheat from the dietary CP digestibility and assuming a soyabean and soya meal digestibility of 82% and 84%, respectively (Valencia et al., 2008). Our calculations showed values ranging from 73.58 to 89.21% (wheats included at 55%) and from 51.33 to 74.35% (wheats at 70%). Ileal dietary CP digestibility was as average 77.92% (Table 3) which indicates an overestimation of the wheat CP digestibility at 55%. Nevertheless, this method is not adequate to estimate with precision the wheat CP digestibility because it does not discriminate among samples.

We did not measure NSP content of the wheat samples. However, the insoluble NSP are included in the fraction NDF and we found a negative correlation between NDF and the ileal and fecal ST digestibility (Table 5). The negative effect of wheat NSP on nutrient digestibility and broiler performance is nowadays widely recognized (Choct and Annison, 1990, 1992) and the use of NSP-degrading enzymes in poultry diets is therefore a common practice. But still there is no evidence that shows that NSP-degrading enzymes will bring back digestibility to the same level of those wheats with different NSP content, equalizing in this way the nutritive value of wheats.

In this experiment, the best equation to predict wheat AMEn showed a determination coefficient of 0.58. This value is not considered high enough to accurately predict AMEn since wheat grains could contribute to approximately 65% of the dietary AMEn (Wiseman et al, 2000). But wheat ST and wheat digestible ST was consistently used in equations and it seems appropriate to study properties of ST itself or properties associated with ST. One extra argument to focus on ST with regard to AMEn is that ST is the energy yielding of the wheat grain (82% of its energy).

In conclusion, in this experiment the prediction of the AMEn of wheat from its physicochemical and ST digestibility properties gave a relatively low determination coefficient (0.58). From the parameters studied, the ST fraction and the factors that affect its digestibility seemed to be of importance.

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CHAPTER 4

Effect of Wheat Cultivar and Enzyme Addition to Broiler Chicken Diets on Performance, Nutrient Digestibility and Apparent Metabolizable Energy Content

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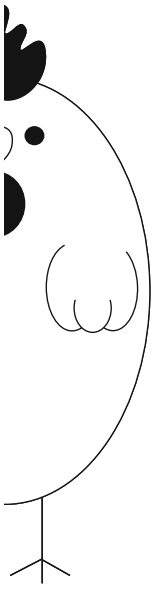

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ABSTRACT A total of 5,000 one-day old male broiler chickens were assigned to 8 different treatments in a 4 x 2 factorial design. Four wheat cultivars (Amiro, Guadalupe, Isengrain and Horzal) and two levels (0 or 1 kg/t feed) of an enzyme cocktail (Avizyme 1300, xylanase, 2,500 U/kg and protease, 800 U/kg) were used. Nutritionally complete mash diets contained 65 and 70% of the test wheat for the starter and grower period, respectively. Test wheats were used in diets for broilers and growth performance and apparent metabolizable energy **(AME)** contents were measured. Broiler performance was measured in 4,800 broilers, allocated in floor pens with 75 birds each and fed from 1 to 42 d of age. Digestibilities and AME content of diets were measured in 200 broilers from 6 to 27 d of age individually allocated in battery cages. Chromic oxide **(Cr_2O_3)** at inclusion rate of 0.5% in the diet was used as indigestible marker. Apparent metabolisable energy was corrected by zero nitrogen balance to obtain AMEn. Wheat cultivar strongly influenced animal performance during the starter period (1 to 21 d of age). During the grower period (21 to 42 d of age) only body weight and daily feed intake were influenced by wheat cultivar. Differences in daily feed intake were associated with differences in AMEn intake during the starter period, but not in the grower period. Nutrient digestibility was higher with the use of enzyme. Animal performance was not affected (i.e., wheat cultivar differences were not eliminated by using enzymes). During the grower period significant interactions were detected with regard to nutrient digestibility and AMEn. Differences in AMEn content of wheat could not be explained by digestible starch.

(Key words: broiler, wheat, metabolisable energy, broiler performance, enzyme)

INTRODUCTION

Wheat is an important ingredient in broiler diets because of its high starch (**ST**) and protein (**CP**) content, and is often the only cereal in grower and finisher diets. However, the chemical composition and energy availability of wheat can vary (Mollah et al., 1983; Kim et al., 2003). In a survey of 18 wheat cultivars, Kim et al. (2003) reported that ST content ranged between 58.5 and 73.7%, CP between 9.7 and 19.1% and non-starch polysaccharides (**NSP**) between 7.8 and 11.0% (on a DM basis). Mollah et al. (1983) found AME ranging between 11.0 and 15.9 MJ/kg DM when 22 wheat samples were fed to growing broiler chickens. Several physical and chemical factors influence wheat AMEn and animal performance, including specific weight (McCracken and Quintin, 2000), viscosity and hardness (Carré et al., 2002), pelleting, (Scott, 2000), ST, CP, NSP, and ether extract content (Steenfeldt, 2001; Svihus and Gullord, 2002; Pirgozliev et al., 2003). However, the relationship between physico-chemical properties of wheat and its AMEn content is not fully defined. Moreover, some wheat cultivars considered to be of high quality have produced unexpectedly low broiler performance.

Most trials measure one or two physico-chemical factors with animals housed in battery cages and fed experimental diets for a limited period of time. In addition, the determination of AMEn in raw materials is commonly done using broilers at one fixed age. Most animals used for testing are less than 20 d old (Scott, 2002; Scott and Silversides, 2003) or slightly older (Carré et al., 2002). Until now, only moderate correlations between wheat AMEn and animal performance have been found (Rose and Bedford, 1995; Steenfeldt, 2001).

Much information exists on the use of NSP degrading enzymes and their beneficial effect on nutrient digestibility (Steenfeldt et al., 1998; Marron et al., 2001; Meng et al., 2005) and on animal performance (Choct et al., 2004; Wang et al., 2005). The use of NSP degrading enzymes in poultry diets is therefore a common practice. However, much less information is available on the efficacy of the enzymes to equalize nutritional values of different wheats. Also the characteristics of the wheats which are improved by the use of the enzymes are not well known.

Our objectives were to determine the effect of wheat cultivar on animal performance and AMEn with or without enzyme addition and to study the relationship between wheat AMEn and performance on animals grown on floor throughout the fattening period.

MATERIAL AND METHODS

Feeds

The experimental design was 4 x 2 with four wheat cultivars (Isengrain, Amiro, Guadalupe and Horzal) and two levels of enzyme addition (0 and 1 kg/t feed). The diets contained high levels of the experimental wheat (65 and 70% in the starter and grower diets, respectively) and met or exceeded broiler requirements (NRC, 1994). The ingredients and the average determined chemical compositions of experimental diets are shown in Table 1. All wheat cultivars used in the study came from the same growing region of Torrijos (Spain). They were selected to have different ST and CP content (Table 2). Wheats were milled separately and passed through a 3 mm screen in a hammer mill (Rosal, 40 CV, Barcelona, Spain) and then mixed with the other ingredients. The exogenous enzyme was a commercial powdered preparation containing xylanase derived from Trichoderma longibrachiatum and protease derived from Bacillus subtilis (Avizyme 1300; EC 3.2.1.8, 2,500 IU and EC 3.4.21.62, 800 IU, respectively). The enzyme was added to the feed by first mixing it with the premix and a small proportion of ground cereal. The amount of enzyme added was based on guaranteed product activities (Danisco Animal Nutrition, Marlborough, UK). In each experimental period, the wheat-based diets were split in two portions. One portion was fed as is, while in the other portion the recommended dose of the commercial powdered enzyme, 1 kg/t feed, was added on top.

Animals and Experimental Design

One-day old male broiler chickens (Ross 308) were obtained from a commercial hatchery (Cazalegas, Toledo, Spain). Animals were randomly assigned to the eight dietary treatments. Feed and water were provided ad libitum during the whole trial. Light was on continuously during the first day and during 16 hours per day afterwards. Broiler chickens were kept at 32ºC during the first wk of age. Thereafter temperatures were decreased with 3ºC per wk to reach 22ºC by 23 d of age. After that, they were maintained around 22ºC until the end of the experiments which was 42 d for the growth assay and 27 d for the metabolic assay.

Experiment 1. Growth assay. A total of 4,800 one-day old male broiler chickens was assigned randomly to 64 pens (7.5 m^2). Each contained 75 broiler chickens. Each pen was equipped with one hanging feeder, a nipple drinker and wood shavings (6cm-deep) as litter. The experimental treatment diets were assigned randomly to 8 pens each. The experimental period lasted 42 d, animals were weighed at the beginning (d 0), when the experimental diets changed from starter to grower (d 21) and at the end, prior to slaughtering (d 42). The chickens were inspected daily and dead birds were removed (date of death and

Table 1. Ingredient profile and determine analysis of starter and grower diets

Ingredient	Starter	Grower
	%	
Wheat	65.00	70.00
Soybean meal	30.45	25.45
Soybean oil	0.75	0.75
Salt	0.25	0.25
Limestone	1.53	1.63
Monocalcium phosphate	1.25	1.25
Sodium bicarbonate	0.22	0.20
L-lysine	-	0.02
L-threonine	0.05	0.05
DL-methionine	0.20	0.10
Mineral-vitamin premix	0.30[1]	0.30[2]
Determined analysis[3]	%	
Dry matter	90.08	90.95
Crude protein	23.43	21.35
Ether extract	2.64	2.43
Ash	6.58	6.38
Starch	41.56	45.27
Crude fiber	3.63	3.55
Neutral-detergent fiber	14.63	14.32
Acid-detergent fiber	5.55	4.97
Lignin	0.90	1.05
Gross energy (kcal/kg DM)	4,307	4,328

[1] Supplied per kg of diet: cobalt , 0.2 mg; copper, 12 mg; iron, 20 mg; manganese, 90 mg; zinc, 59 mg; selenium, 0.2 mg; iodine, 2 mg; vitamin A, 9,999 IU; vitamin E, 37 IU; cholecalciferol, 75 µg; vitamin B_1, 2 mg; vitamin B_2, 12 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.015 mg; vitamin K, 4 mg; pantothenate, 12 mg; folic acid, 1.5 mg; niacin, 50 mg; biotin, 0.15 mg; choline, 350 mg; B.H.A plus ethoxyquine, 100 mg; narasin, 8 mg; nicarbacin, 8 mg.

[2] Supplied per kg of diet: cobalt , 0.2 mg; copper, 8 mg; iron, 20 mg; manganese, 80 mg; zinc, 59 mg; selenium, 0.2 mg; iodine, 2 mg; vitamin A, 9,999 IU; vitamin E, 29 IU; cholecalciferol, 50 µg; vitamin B_1, 2 mg; vitamin B_2, 8 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 0.01 mg; vitamin K, 3 mg; pantothenate, 10 mg; folic acid, 1 mg; niacin, 50 mg; biotin, 0.1 mg; choline, 250 mg; B.H.A plus ethoxyquine, 100 mg; salinomycin, 7.2 mg.

[3] Mean values of the 8 experimental diets.

body weight were recorded). To calculate feed conversion ratio **(FCR)**, the body weight **(BW)** of dead birds was considered. Sixteen animals per treatment on d 7 and 8 animals per treatment on d 13, 20, 27 and 34 were randomly selected and euthanasied following the principles for care of animals in experimentation (Spanish Royal Decree 1201/2005, 2005). Immediately after, the small intestine was removed. The ileum, defined as the area between the Meckel's diverticulum and the ileocaecal junction was dissected and the digesta from this area was collected by gentle finger-stripping. After collection, the samples were immediately centrifuged at 4,500 rpm for 10 minutes (Mixtasel, Selecta, Barcelona, Spain), the supernatant collected and ileal viscosity measured.

Table 2. Chemical and physical properties of the different wheat[1] cultivars

Wheat cultivar	Isengrain	Amiro	Guadalupe	Horzal
Parameter[2]	% DM			
Starch	68.45	67.46	67.57	66.68
Crude protein	9.01	9.94	10.94	13.71
Crude fiber	3.13	2.93	3.21	2.82
Ether extract	1.78	1.68	1.80	1.73
Ash	1.73	1.85	2.01	1.57
NDF	15.26	17.06	17.30	16.85
ADF	3.72	3.80	4.41	3.52
Lignin	0.59	0.87	0.87	0.65
Total NSP	9.76	10.79	11.23	10.91
Total soluble NSP	1.33	2.40	2.04	2.41
Arabinose	0.35	0.66	0.65	0.76
Xylose	0.46	1.04	0.88	1.04
Total insoluble NSP	8.42	8.39	9.19	8.51
Arabinose	2.00	1.96	2.34	2.22
Xylose	2.95	3.30	3.34	3.20
Gross energy (kcal/kg)	4,368	4,438	4,407	4,469
Viscosity (cp)	4.98	5.89	6.47	4.53
Specific weight (kg/hl)	74.40	72.70	77.40	81.10
P (mm)	57.00	63.00	76.00	123.00
L (mm)	18.00	43.00	24.00	39.00
P/L	3.16	1.46	3.16	3.15
W (10-4 J)	49	102	84	222

[1] Measured in the wheat grain except P,L and W that was measured in the wheat flour.

[2] NDF, neutral-detergent fiber; ADF, acid-detergent fiber; NSP, non-starch polysaccharides; P, tenacity or extensibility resistance; L, extensibility; P/L, index of gluten behavior; W, baking strength.

Experiment 2. Metabolic assay. Two hundred one-day old male broiler chickens were housed in the same room as in Experiment 1 and fed on a mash starter diet for the first wk. At d 6 all birds were weighed, 80 of them were selected for the experiment (BW 101 ± 16 g) and moved to another room prepared with metabolic cages (19.5 x 38.5 x 35 cm, width x length x height). Animals were randomly assigned to the 8 dietary treatments and individually allocated to the cages (10 replicates / treatment). This allowed total collection of excreta from each individual separately. Each cage was fitted with a metal feeder and a drinker.

Experimental diets were the same as in Experiment 1, except that 0.5% of chromic oxide **(Cr_2O_3)** was added and mixed to facilitate determination of nutrient digestibility. The experimental period lasted 21 d (from d 6 to d 27). Excreta was collected for 3 consecutive days, from d 10 (BW 177 ± 29 g) to d 13 for the starter feed and from d 24 (BW 694 ± 97 g) to d 27 for the grower feed. Contaminations, such as down and feathers, were carefully removed and the excreta was stored in containers at –20ºC. Afterwards, excreta was dried at 70 ± 0.5ºC during 48 hours. Samples collected from each bird during the 3 d of excreta collection were blended, ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored in plastic tins until analysis. Samples were analyzed for ST, nitrogen (**N**) and gross energy (**GE**) to determine ST digestibility, N retention and AME. The AME was corrected to zero N balance (**AMEn**) according to Hill and Anderson (1958).

At the end of the experiment (d 27) all birds were killed by cervical dislocation and immediately afterwards the small intestine was removed. The ileal content was collected as explained previously and it was freeze-dried. Ileal contents of two animals from the same treatment were pooled, ground through a 0.75 mm sieve and stored in plastic bags until analysis. Samples were analyzed for ST, N and chromium.

Chemical Analysis

All analysis, except Cr_2O_3 in feed (9 times/treatment), were carried out on duplicate and results reported on DM basis. Chemical analysis of the wheats, diets and excreta were conducted according to the methods of AOAC (1995) for DM (930.15), N (954.01), crude fiber (962.09), ether extract (960.39) and ash (942.05). Neutral-detergent fiber, acid-detergent fiber and acid-detergent lignin were determined sequentially following the procedures described by Van Soest et al. (1991). Starch content was analyzed following the alpha-amylogluclosidase method (996.11). Total, soluble and insoluble NSP and their constituent sugars were determined using the method described by Bach Knudsen (1997) with the exception that the polysaccharides in starch free residues were treated with 12 mol/L H_2SO_4 and hydrolyzed to monosaccharides with 2 mol/L (100ºC, 60 min) instead of with 1 mol/L (100ºC, 120 min). Physical properties of the wheat flour were determined according to the method of the ICC (1998) for alveograph (121) using the Chopin alveograph

model MA82 (Chopin technologies, France). Chromium oxide content in feed and excreta was determined according to Fenton and Fenton (1979). Ileal viscosity was measured according to Bedford and Classen (1993). Gross energy values were determined by bomb calorimeter using Parr 6100 adiabatic calorimeter (Parr Instrument Company, Moline, IL, USA).

Calculations and Statistical Analysis

In the metabolic assay, the following equations were used for calculation of apparent total tract digestibility (using ST digestibility as an example), apparent ileal digestibility and AMEn content of experimental diets (Hill and Anderson, 1958):

$$\text{Apparent total tract ST digestibility (\%)} = \{1 - [(Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ excreta}) \times (ST_{\%\ excreta} / ST_{\%\ diet})]\} \times 100,$$

$$\text{Apparent ileal ST digestibility (\%)} = \{1 - [(Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ digesta}) \times (ST_{\%\ digesta} / ST_{\%\ diet})]\} \times 100,$$

$$\text{AMEn (kcal/kg of diet)} = GE_{kcal/kg\ diet} - [GE_{kcal/kg\ excreta} \times (Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ excreta})] - 8.22 \times \{N_{\%\ diet} - [N_{\%\ excreta} \times (Cr_2O_{3\ \%\ diet} / Cr_2O_{3\ \%\ excreta})]\}$$

where GE is gross energy, Cr_2O_3 is chromic oxide, ST is starch, N is nitrogen and 8.22 is the energy equivalent of 1 g of uric acid N.

Data from growth and metabolic assay were analyzed with ANOVA using the GLM procedure of SAS software (SAS Institute, 1985). The factors were 4 wheat cultivars and 2 levels of enzyme. If significant interaction existed between main effects, the data were reanalyzed using one-way ANOVA. Mortality data were transformed into arcsine of the square root for statistical analysis, but the data are presented as natural numbers. Viscosity was analyzed using one-way ANOVA repeated measurements. Means were separated by using Duncan's multiple test. Pooled SEM were calculated from the mean square error term generated by one-way ANOVA. Relationships between wheat characteristics and chicken performance and AMEn of the diets were estimated using a simple Pearson correlation analysis. All statements of significance were based on a probability of equal or less than 0.05.

RESULTS

Growth Assay

The effect of the wheat cultivar and enzyme addition on BW, daily gain **(DG)**, daily feed intake **(DFI)**, FCR and mortality at d 21 and d 42 are shown in Table 3. The wheat cultivar had a clear effect on most broiler growth parameters. At d 21 birds fed diets containing Isengrain cultivar had the greatest (622 g, $P < 0.001$) BW followed by the animals fed diets containing Guadalupe (603 g), Amiro and Horzal (average 580 g). The differences in BW due to wheat cultivar at d 42 were lower (maximum differences of 3.7 %) than at d 21, but wheat cultivar ranking was maintained. Daily feed intake was greater ($P \leq 0.03$) for animals fed Guadalupe than for those fed Amiro and Horzal cultivars in both, starter (42.06 vs. 40.55 g/d, respectively) and grower period (89.00 vs. 86.55 g/d, respectively) while those fed Isengrain showed intermediate values (41.21 and 88.11 g/d for starter and grower period, respectively). The FCR at d 21 was lower ($P \leq 0.001$) for birds fed the Isengrain cultivar (1.490) than for those fed any other wheat cultivar (average 1.573). At d 42 there was no effect of wheat cultivar on FCR. The average value was 1.933. None of the dietary treatments affected mortality in any of the periods.

The addition of a NSP enzyme cocktail did not have a clear effect on growth performance under conditions tested (Table 3). In the grower period, DFI was greater ($P = 0.037$) in chicks fed diets without enzyme (131 g/d) than those fed enzyme-supplemented diets (129 g/d).

Intestinal viscosity of chicks fed the experimental diets decreased with age (range from 11.90 to 2.83, $P \leq 0.03$, Figure 1a). The reduction was most pronounced between d 7 and d 13. Dietary exogenous enzyme cocktail reduced ($P \leq 0.001$) the intestinal viscosity during the whole period, up to 60 % at d 7 (Figure 1a). No correlations between intestinal viscosity and animal performance were found except at d 7, which affected BW at d 21 ($r = -0.31$, $P = 0.018$) and DG during the starter period ($r = -0.30$, $P = 0.018$) (data not shown). Wheat cultivar clearly affected intestinal viscosity at the ileum. Horzal cultivar had the greatest viscosity at all ages (average 6.90 cp) and Guadalupe and Isengrain the lowest (average 5.52 and 4.19 cp, respectively). An interaction between enzyme addition and wheat cultivar was found for animals at d 20 ($P \leq 0.01$, Figure 1b) and d 27 ($P \leq 0.03$). Enzyme cocktail supplementation to Horzal cultivar reduced ileal viscosity to a greater extent compared to the other wheat cultivars.

Metabolic Assay

Daily feed intake between treatments was in the same range as reported in the growth assay for both starter (average 40.53 ± 6.9 g/d) and grower period (average 92.85 ± 18.5 g/d).

Table 3. Effect of wheat cultivar and enzyme[1] addition[2] on broiler performance (mean values)

	Starter period (d 1 to d 21)[4]					Grower period (d 21 to d 42)[4]
Effect[3]	BW-21	DG g	DFI g/d	FCR g/d	Mort. %	BW-42 g
Isengrain						
-	618	27.46	41.11	1.497	3.00	2,046
+	625	27.83	41.30	1.484	1.33	2,042
Amiro						
-	586	25.95	40.24	1.551	2.66	2,015
+	581	25.72	40.04	1.557	2.00	1,979
Guadalupe						
-	596	26.44	42.24	1.598	3.00	2,019
+	609	27.04	41.88	1.549	3.16	2,049
Horzal						
-	570	25.18	39.53	1.571	2.66	1,978
+	580	25.67	41.46	1.618	3.16	1,962
Pooled SEM	8.06	0.38	0.67	0.02	0.68	19.89
Main effect mean[5]						
Wheat cultivar						
Isengrain	622^{a}	27.65^{a}	41.21^{ab}	1.490^{b}	2.16	2,044^{a}
Amiro	584^{c}	25.84^{c}	40.14^{b}	1.554^{a}	2.33	1,997^{bc}
Guadalupe	603^{b}	26.74^{b}	42.06^{a}	1.573^{a}	3.08	2,033^{ab}
Horzal	575^{c}	25.42^{c}	40.96^{b}	1.594^{a}	2.91	1,970^{c}
Enzyme						
-	593	26.26	40.78	1.554	2.83	2,014
+	599	26.56	41.17	1.552	2.41	2,008
Statistic probability						
Wheat cultivar	< 0.001	< 0.001	0.032	< 0.001	0.501	< 0.001
Enzyme addition	0.262	0.264	0.421	0.903	0.171	0.639
Wheat cult. x ez. addi.	0.711	0.705	0.318	0.256	0.392	0.409

$^{a-c}$ Means within a given column with no common superscript are significantly different (P < 0.05).

[1] Avizyme 1300. [2] 0 (-) or 1 kg/t feed (+). [3] Each mean represents 8 pens with 75 animals each.

				Overall period (d 1 to d 42)[4]			
DG g/d	DFI g/d	FCR	Mort. %	DG g/d	DFI g/d	FCR	Mort. %
68.00	132.90	1.955	1.46	47.73	89.00	1.865	4.33
67.44	129.57	1.921	1.60	47.63	87.22	1.831	2.83
68.04	132.63	1.949	1.45	47.00	88.33	1.879	4.00
66.56	128.30	1.929	0.72	46.14	86.02	1.865	2.66
67.74	131.07	1.934	0.72	47.08	88.49	1.879	3.66
68.56	133.21	1.943	0.90	47.80	89.50	1.872	4.00
67.07	128.41	1.915	0.36	46.12	85.91	1.863	3.00
65.80	126.36	1.922	1.45	45.74	85.99	1.882	4.50
0.78	1.25	0.68	0.38	0.47	0.81	0.01	0.76
67.72	131.23^{a}	1.938	1.53	47.68^{a}	88.11^{ab}	1.848	3.58
67.30	130.46^{a}	1.939	1.08	46.57^{bc}	87.17^{bc}	1.872	3.33
68.15	132.14^{a}	1.938	0.81	47.44^{ab}	89.00^{a}	1.875	3.83
66.43	127.38^{b}	1.918	0.90	45.93^{c}	85.94^{c}	1.872	3.75
67.71	131.25	1.938	1.00	46.98	87.93	1.871	3.75
67.09	129.36	1.929	1.16	46.83	87.18	1.862	3.50
0.171	0.002	0.581	0.177	0.001	0.003	0.306	0.947
0.268	0.037	0.433	0.352	0.637	0.200	0.435	0.731
0.456	0.062	0.553	0.387	0.407	0.153	0.447	0.311

[4] BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion rate; Mort. = mortality.

[5] Each mean represents 16 and 32 pens with 75 animals each, for wheat cultivar and enzyme addition, respectively.

Table 4. Effect of wheat cultivar and enzyme[1] addition[2] on excreta digestibility and AMEn of broilers (

Starter period (10-13 days of age)[4]

Effect[3]	DM %	ST %	CP ret. %	AMEn kcal/kg DM
Isengrain				
-	70.76	99.26	62.41	2,998
+	70.90	99.33	60.28	3,021
Amiro				
-	70.24	99.13	60.52	2,982
+	71.76	99.53	61.19	3,058
Guadalupe				
-	68.60	99.29	60.40	2,886
+	68.87	99.40	60.42	2,868
Horzal				
-	68.55	98.51	56.32	2,936
+	70.39	99.40	58.18	3,014
Pooled SEM	0.60	0.23	0.86	26.61
Main effect mean[5]				
Wheat cultivar				
Isengrain	70.83^{ab}	99.29	61.29^{a}	3010^{a}
Amiro	71.04^{a}	99.34	60.83^{a}	3022^{a}
Guadalupe	68.76^{c}	99.35	60.41^{a}	2876^{b}
Horzal	69.63^{bc}	99.03	57.41^{b}	2982^{a}
Enzyme				
-	69.64	99.06	59.99	2,954
+	70.45	99.41	60.09	2,989
Statistic probability				
Wheat cultivar	< 0.001	0.334	< 0.001	< 0.001
Enzyme addition	0.034	0.033	0.865	0.039
Wheat cult. x enzyme addit.	0.397	0.303	0.136	0.241

$^{a-d}$ Means within a given column with no common superscript are significantly different (P < 0.05). [1] Avizyme 1300. [2] 0 (-) or 1 kg/t feed (+). [3] Each mean represents 10 cages with one animal each. [4] DM, dry matter; ST, starch; CP ret.,

(mean values)

Grower period (24-27 days of age)[4]

DM %	ST %	CP ret. %	AMEn kcal/kg DM
72.23^{ab}	98.46	61.10	3,066^{ab}
72.63^{a}	99.25	59.48	3,077^{ab}
69.81^{bcd}	97.61	58.42	3,005^{bc}
70.80^{abc}	98.51	58.99	2,996^{bcd}
69.70^{bcd}	98.97	57.76	2,961^{cd}
68.29^{cd}	98.49	58.01	2,902^{d}
67.34^{d}	97.94	54.09	2,913^{cd}
71.68^{ab}	98.97	57.26	3,108^{a}
0.84	0.45	1.28	32.56
72.44^{a}	98.88	60.24^{a}	3,071^{a}
70.33^{b}	98.08	58.72^{a}	3,000^{b}
68.98^{b}	98.72	57.89^{ab}	2,930^{c}
69.62^{b}	98.48	55.75^{b}	3,015^{ab}
69.78	98.26	57.82	2,985
70.45	98.81	58.42	3,021
< 0.001	0.330	0.005	< 0.001
0.079	0.088	0.521	0.138
0.008	0.319	0.316	0.001

crude protein retention.; AMEn, apparent metabolisable energy of the diets corrected by zero nitrogen retention. [5]

Each mean represents 20 and 40 cages with one animal each, for wheat cultivar and enzyme addition, respectively.

Digestibility coefficients and AMEn content of the diets are presented in Table 4. Digestibility of DM, protein retention **(PR)** and AMEn of the diets in both starter and grower period were affected by wheat cultivar ($P < 0.05$). During the starter period DM digestibility of the diets including Isengrain and Amiro cultivars was greater (average 70.93%) than in the diets with the other cultivars (average 69.19%). During the grower period Isengrain cultivar showed the greatest DM digestibility (72.44%). In both periods the digestibility of ST was very high (99.23 and 98.53% for starter and grower period, respectively) and not influenced by wheat cultivar.

Protein retention during the starter period was lower ($P \leq 0.001$) for diets with Horzal cultivar (57.41%) than for the other cultivars (average 60.84%). During the grower period, PR was similar than during the starter period (1% less) but differences among cultivars remained.

The diet which contained Guadalupe cultivar showed the lowest ($P < 0.001$) AMEn during both the starter (2,876 kcal/kg DM) and the grower period (2,930 kcal/kg DM). During the grower period Isengrain and Amiro cultivars differed ($P < 0.001$) by 71 kcal/kg DM (3,071 vs. 3,000 kcal/kg DM, respectively) whereas Horzal cultivar presented an intermediate value (3,015 kcal/kg DM).

The addition of an exogenous enzyme cocktail increased ($P = 0.03$) the digestibility of the DM, ST and AMEn of the diets by 1 % in the starter but not in the grower period (Table 4). During the grower period an interaction between wheat cultivar and enzyme supplementation was found for DM digestibility ($P = 0.008$) and AMEn ($P < 0.001$) of the diets. Enzyme supplementation increased excreta DM digestibility (from 67.3 to 71.6 %) and AMEn (from 2,913 to 3,108 kcal/kg DM) in diets based on Horzal cultivar but not in those based on the other cultivars. There were no differences among wheat cultivars (Table 5) for ileal DM, ST and protein digestibilities (average 70.8, 97.9 and 79.5%, respectively). The addition of the exogenous enzyme cocktail did not increase ileal digestibilities.

DISCUSSION

According to our results, wheat cultivar type can affect animal performance and nutrient availability and should be taken into account in diet formulations. Daily feed intake was affected by wheat cultivar in both the starter and grower periods but it was not fully related with wheat AMEn. Energy consumption during the starter and DFI during the grower period were the parameters mainly related to DG. Scott (2000, 2002), observed that DG is better explained by feed intake than by AMEn in animals allocated in cages from 4 to 17 d of age. This agrees with our data. Nevertheless, none of the wheat physico-chemical parameters or diet digestibilities analyzed were highly related to DFI, in our study with four wheat cultivars.

Table 5. Effect of wheat cultivar and enzyme[1] addition[2] on ileal digestibility[3] of broilers at d 27 (mean values)

Effect[4]	DM %	ST %	CP %
Isengrain			
-	71.6	98.3	78.5
+	70.6	97.7	81.3
Amiro			
-	69.9	98.6	77.8
+	69.5	96.5	79.2
Guadalupe			
-	69.3	97.6	79.3
+	74.5	99.0	81.5
Horzal			
-	70.4	97.4	78.9
+	70.6	98.3	79.5
Pooled SEM	1.10	0.59	0.99
Main effect mean[5]			
Wheat cultivar			
Isengrain	71.2	98.0	79.7
Amiro	69.7	97.4	78.5
Guadalupe	71.5	98.2	80.4
Horzal	70.5	97.9	79.2
Enzyme			
-	70.3	98.0	78.6
+	71.2	97.8	80.4
Source of variation			
Wheat cultivar	0.63	0.78	0.57
Enzyme addition	0.37	0.77	0.09
Wheat cult. x enzyme	0.20	0.17	0.87

[1] Avizyme 1300.

[2] 0 (-) or 1 kg/t feed (+).

[3] DM = dry matter; ST = starch; CP = crude protein.

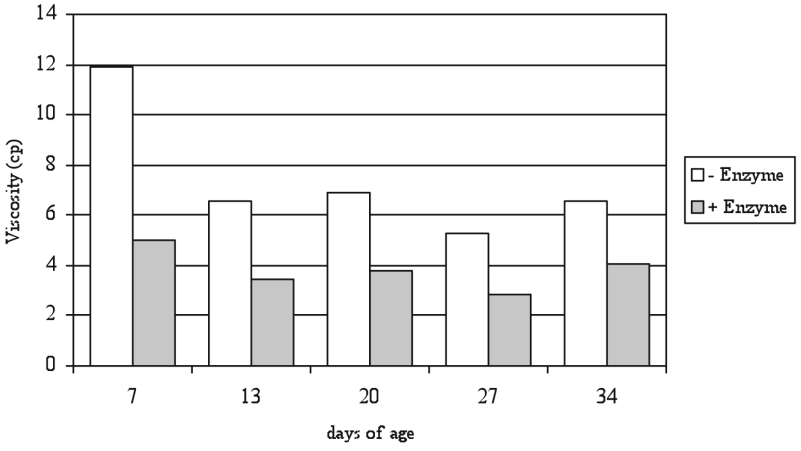
[4] Each mean represents 5 ileal samples of two broilers each.

[5] Each mean represents 10 and 20 ileal samples of two broilers each, for wheat cultivar and enzyme addition, respectively.

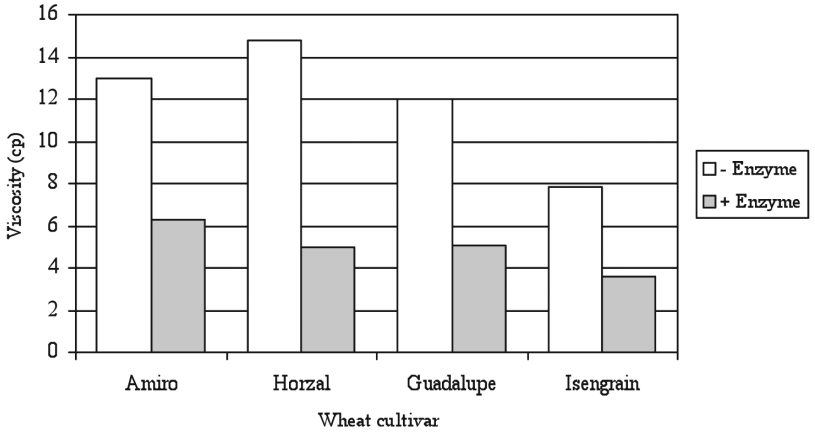
Excreta digestibility of DM, N retention and AMEn of the diets were affected by wheat cultivar, contrary to the ileal digestibilities analyzed. Starch digestibility measured with excreta was almost 100% for all cultivars. Starch is the most important energy yielding nutrient in wheat grain and which contributes most to its AMEn. In this study, the differences among wheat cultivars in starch content were low (2.6%), but those in

Figure 1. Digesta viscosity (centipoises; cp) of ileal contents[1] from broilers fed with diets containing different wheat cultivars and supplemented with enzyme[2].

a. Evolution as bird ages



b. at d 20 of age



[1] *In figure a and b, each mean represents 32 and 8 replicates, respectively.*

[2] *Avizyme 1300 added at 0 (-) or 1 kg/t feed (+).*

dietary AMEn were about 5%, and there was no relationship between total ST or ST digestibility and AMEn content. In a study (Mollah et al., 1983) with 22 samples of wheat where ST content ranged from 59 to 72%, there was no relationship between ST and AMEn. However, in the same study, ST digestibility was different among wheat cultivars (from 80 to 99%) and correlated with AMEn. The amount of digestible ST is what most contributes to AME (Longstaff and McNab, 1986; Wiseman, 2006). This contribution ranged from 73 to 79% in the current study, but its variation explained less than 22% of the variation in dietary AMEn. When diets were non enzyme supplemented, dietary AMEn mainly related to the total NSP content of the wheat cultivars ($r = -0.42$, $r = -0.53$, $P < 0.02$, for starter and grower period, respectively). However, dietary AMEn mainly related to the insoluble NSP content ($r = -0.7$, $r = -0.47$, $P < 0.05$) in enzyme supplemented diets.

The beneficial effect of the use of exogenous enzymes in the present study was observed only in some variables, and depended on the wheat cultivar used. Daily feed intake was not increased in animals fed enzyme supplemented diets. This indicates that enzymes cannot eliminate the differences in DFI among wheat cultivars. This is in concordance with Scott et al. (1998), who observed that DFI variability among animals fed different wheat cultivars could not be eliminated by enzyme supplementation although its addition increased DFI.

In our study, enzyme addition increased AMEn in animals fed the Horzal cultivar. Xylanase reduces intestinal viscosity in birds by degrading soluble NSP arabinoxylans (McCracken et al., 1999; Choct et al., 2004). Horzal cultivar had the highest concentration of soluble arabinose. When this cultivar was enzyme supplemented, the reduction in the intestinal viscosity was larger than in the other cultivars (Figura 1b). Choct et al. (2004) used different enzymes on two wheat cultivars with low and normal-ME. The enzymes that reduced ileal viscosity increased the AME value of the low-ME wheat. This may indicate that xylanase supplementation is most effective when intestinal viscosity is high, while there is no or little effect at medium-low viscosity values. The effect of enzyme addition was also related to the CP content of wheat ($r = 0.52$ to 0.79) and also to the baking strength ($r = 0.74$ to 89). The latter represents physical protein behavior, which can be explained by the protease activity present in the enzyme used.

In conclusion, wheat cultivar affects feed intake and animal performance and AMEn does not appear to be involved in this effect. Based on the wheat cultivars used in this study, digestible ST does not explain the variations in AMEn between wheat cultivars, but no other important relationship with chemical composition was found. Further, the use of an exogenous enzyme did not eliminate the differences between cultivars, as its effect was cultivar dependent.

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CHAPTER 5

Wheat Starch Digestion Rate in Broiler Chickens is Affected by Cultivar but not by Wheat Crop Nitrogen Fertilization

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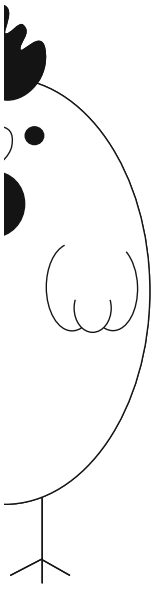

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ABSTRACT A study was set up to investigate the influence of wheat cultivar and wheat crop nitrogen (**N**) fertilisation on starch (**ST**) digestion rate in broiler chickens. A total of 288 broiler chickens were used in a 3 x 2 factorial design with diets based on three varieties of wheat (Apache, Caphorn and Charger) each grown at two N fertilisation levels (40 and 170 kg of N/ha). Starch digestion rate was determined by measuring the remaining starch and the mean retention time **(MRT)** in four segments of the small intestine (proximal and distal jejunum and proximal and distal ileum) and in excreta, using chromic oxide as a marker. Varietal differences in starch content (i.e. 71.4 to 74.6%) were smaller than differences caused by crop N fertilisation (70.5 to 75.5%). Nitrogen fertilisation increased wheat crude protein **(CP)** content from 9.4 to 13.0%. The majority of the ST in all diets was digested by the time the digesta reached the distal ileum (average 84% in the distal jejunum and 96% in the proximal ileum). Starch digestion differed among wheat cultivars in the proximal jejunum (from 43 to 57%, $P<0.001$) with no differences due to wheat cultivar or N fertilisation found afterwards. Starch digestion rate varied among wheat cultivars (from 2.45 to 3.28 h^{-1}, $P<0.001$), but did not vary with N fertilisation, whereas dietary CP digestion rate was not affected by wheat cultivar or N fertilisation level. The digestion rate of ST was faster than that of CP (average 2.78 vs 1.53 h^{-1}). The current study suggests that wheat cultivars can be classified based on their rate of ST digestion independently of the N fertilisation applied to the crop during growth.

(Key words: broiler, wheat, starch digestion rate, cultivar, fertilisation)

INTRODUCTION

Wheat grain is the main energy source used in European broiler diets with inclusion percentages up to 55-60%. Digestible starch **(ST)** is what most contributes to the apparent metabolizable energy **(AME)** of wheat (Longstaff and McNab, 1986; Wiseman, 2006), supplying up to 79% of the dietary nitrogen-corrected apparent metabolizable energy **(AMEn)** (Gutierrez del Alamo et al., 2008). However, wheat AMEn is not consistently correlated to digestible ST (only 22% of the dietary AMEn variation was explained by its digestible ST, Gutierrez del Alamo et al., 2008) nor to any other chemical or physical (i.e., hardness, viscosity, specific weight, particle size) properties of the wheat grain (Wiseman, 2000; Steendfeldt, 2001; Carré et al., 2002; McCracken et al., 2002; Svihus and Gullord, 2002). Moreover, when different wheat samples were fed to broiler chickens, Scott et al. (1998) and Gutierrez del Alamo et al. (2008) found differences in animal performance not explained by either total ST digestibility or AMEn. Therefore, it seems that there are some factors, not controlled until now, that influence ST utilization and animal performance.

In a review, Wiseman et al. (2000) suggested that the rate of ST hydrolysis defines wheat AME better than the ST hydrolysed at one specific time. Later, Weurding et al. (2001) found differences in ST digestion rate (from 0.55 h^{-1} (55% of the total ST digested within one hour) for raw potato ST, to 4.3 h^{-1} for tapioca) when 12 ST sources were fed to broiler chickens. In their experiment only one wheat sample was used (considered as rapidly digestible ST, 2.5 h^{-1}). Therefore it would be interesting to determine if different wheat cultivars will have different rate of ST digestion, independently of its total ST digestibility.

In addition, upon analysis of the data of Weurding et al. (2001) a negative relationship between protein content of the different ST sources and ST digestion rate seemed to occur. In cereal grains an accessibility problem has been hypothesized as a factor that reduces ST digestibility (Hesselman and Åman, 1986; Carré et al., 2002). The ST in the wheat grain is stored as granules, located in the endosperm and embedded in a protein matrix (Hoseney, 1998). The specific composition of the proteins of the endosperm is genetically determined (Wrigley et al. 1982; Carrillo et al. 1988) but the protein content depends also on growing conditions, mainly nitrogen **(N)** fertilization (Uhlen et al., 2004). Therefore, the increase in wheat crude protein **(CP)** content, produced by N fertilisation, could affect the ST digestion.

The objectives of the present trial were to determine the ST digestion rate of different wheat samples analysing its variation due to wheat cultivar and N fertilisation level in the wheat crop.

MATERIAL AND METHODS

Wheat cultivars and feeds

Three varieties of wheat (Apache, Caphorn and Charger) were grown at two N fertilisation levels (N1, 40 kg of N/ha and N2, 170 kg of N/ha) in the same place (Research Station of ARVALIS-Institut du végétal, France). Therefore, six different wheat samples were used in this study (Table 1).

Table 1. Chemical and physical characteristics of the different wheat samples

Wheat cultivar	Apache		Caphorn		Charger	
N fertilisation	N1	N2	N1	N2	N1	N2
	%					
Starch	77.36	71.88	74.75	68.11	74.54	72.55
Crude protein	9.61	13.60	9.97	13.43	8.79	12.16
Crude fiber	3.04	2.62	2.85	2.68	2.61	2.50
Ether extract	1.68	1.51	1.62	1.52	1.59	1.58
Ash	1.86	1.72	1.70	1.74	1.76	1.74
NDF[1]	12.95	14.28	13.18	13.09	15.37	15.19
ADF[1]	3.87	3.36	4.13	4.35	3.75	4.31
Lignin	1.00	0.82	1.14	1.24	0.99	1.32
Gross energy (kcal/kg DM)	4,302	4,374	4,350	4,375	4,350	4,350
Viscosity (cp)	5.16	8.18	7.86	10.2	3.92	4.66

[1] *NDF, neutral-detergent fiber; ADF, acid-detergent fiber.*

Each wheat sample was incorporated into a diet formulated to meet or exceed broiler chickens nutrient requirements (NRC, 1994). Diets were composed of wheat (54.7%), full-fat soya bean (26.9%), soya bean meal-47 (11.2%), soya oil (2.4%), synthetic amino acids[1] (0.4%), minerals[2] (0.36%) and the vitamin-mineral premix[3] (0.3%). Chromic oxide ($\mathbf{Cr_2O_3}$, 0.5%) was included as an indigestible marker. Wheats were milled separately and passed through a 2.5 mm screen in a hammer mill (Rosal, 40 CV, Barcelona, Spain) and then mixed with the other ingredients to constitute the experimental diets. Diets were presented as a mash and offered ad libitum to the chickens.

[1] DL-methionine, 2.2 g/kg and L-lysine, 1.8 g/kg.

[2] Monocalcium phosphate, 11 g/kg; salt, 3 g/kg; limestone, 20 g/kg; sodium bicarbonate, 1.8 g/kg.

[3] Supplied per kg of diet: cobalt , 0.2 mg; copper, 8 mg; iron, 20 mg; manganese, 80 mg; zinc, 59 mg; selenium, 0.2 mg; iodine, 2 mg; vitamin A (retinol), 3.4 mg; vitamin E (alpha-tocopherol), 29 mg; vitamin D3 (cholecalciferol), 50 µg; vitamin B1 (thiamine), 2 mg; vitamin B2 (riboflavin), 8 mg; vitamin B6 (pyridoxine), 1.5 mg; vitamin B12 (cyanocobalamin), 0.01 mg; vitamin K3 (menadione), 3 mg; vitamin B5 (pantothenic acid), 10 mg; vitamin B9 (folic acid), 1 mg; vitamin B3 (niacin), 50 mg; vitamin B7 (biotin), 0.1 mg; choline, 250 mg; B.H.A plus ethoxyquine, 100 mg; salinomycin, 72 mg.

Experimental Design and Animals

In an in vivo experiment, ST and CP digestion rate of the 6 experimental diets were analysed. Starch in wheat was the only ST source in the experimental diets. On the contrary, the CP was provided by wheat, soya bean and synthetic amino acids, although the variation among diets is only attributed to wheat samples.

The experimental design was 3 x 2 with three wheat cultivars (Apache, Caphorn and Charger) and two N fertilisation levels (N1 and N2). A total of 400 chickens were obtained from a commercial hatchery (Cazalegas, Toledo, Spain). Animals were fed on a mash standard diet covering all nutrient requirements (NRC, 1994) until d 21. At d 21, chickens were weighed individually and 288 of them with similar body weight (BW 703 ± 46 g) were selected for the experiment. Animals were moved to another room with metabolic cages (19.5 x 38.5 x 35 cm, width x length x height) where ambient temperature and light were controlled (22ºC and 16 h light/day). Chickens were placed individually in battery cages at random. Each experimental treatment consisted of 8 replicates, each with six cages (experimental unit).

Sampling for Digestion Rate Determinations

Feed intake **(FI)** was monitored from d 21 until the end of the experiment at d 30. The average FI of the last 3 days of the experimental period (d 27 to d 30) was used to calculate the mean retention time **(MRT)**.

Excreta was collected per replicate for 3 consecutive days, from d 27 to d 30. Contaminations, such as down and feathers, were carefully removed and the excreta was stored in containers at –20ºC. Afterwards, excreta was dried at 70 ± 0.5ºC during 48 hours. Samples collected from each replicate (6 chickens) during the 3 d of excreta collection were blended, ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored in plastic tins until analysis.

At the end of the experiment (d 30) all birds were euthanasied by cervical dislocation following the principles for animals in experimentation (Spanish Royal Decree 1201/2005, 2005). Immediately afterwards, the small intestine was removed, the mesenterium was cut, and jeju-

num and ileum were separated at Meckel's diverticulum. Jejunum and ileum contents were collected as described by Weurding et al. (2001). In this procedure, both jejunum and ileum are each split into two parts of equal length (proximal jejunum, **PJ**; distal jejunum, **DJ**; proximal ileum, **PI** and distal ileum, **DI**). Digesta are rinsed out of each segment (without squeezing) by using demineralised water at 4ºC. Digesta were collected separately by segment (PJ, DJ, PI and DI) and replicate (6 chickens) and stored at –80ºC and freeze-dried. After freeze-drying, the samples were ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored in plastic bags until analysis.

Analysis

All analysis, except Cr_2O_3 in feed (8 times/treatment), were carried out in duplicate and the results are expressed on a DM basis. Chemical analysis of the wheats, diets and excreta were conducted according to the methods of AOAC International (1995) for dry matter (DM, 930.15), N (954.01), crude fiber (962.09), ether extract (960.39) and ash (942.05). Neutral-detergent fiber, acid-detergent fiber and acid-detergent lignin were determined sequentially following the procedures described by Van Soest et al. (1991). Starch content was analysed following the alpha-amylogluclosidase method (996.11, AOAC International). Viscosity of the grains was measured according to Bedford and Classen (1993). Chromium oxide content in feed and excreta was determined according to Fenton and Fenton (1979). Gross energy values were determined by bomb calorimeter using Parr 6100 adiabatic calorimeter (Parr Instrument Company, Moline, IL, USA). Dry sieve analyses of diets were performed to check whether particle size distributions were similar for all diets (Pfost and Headley, 1976).

Calculations and Statistical Analysis

Apparent digestibility coefficients of ST and CP were calculated in each intestinal segment (PJ, DJ, PI, DI) and in the total tract by calculating the ratio Cr_2O_3 concentration in the feed and digesta or excreta. AME and AMEn of the diets were calculated according to Hill and Anderson (1958).

The pattern of ST and CP disappearance (Ds or Dp, respectively) with the digestion time (t) was described for each replicate using the model proposed by Ørskov and McDonald (1979):

$$Ds = D \times (1-e^{-kd \times t})$$

where D represents the amount of potential digestible ST (or CP) which is digested at a rate of kd (h^{-1}). Starch and protein absorption was assumed not to take place prior to the small

intestine. Due to endogenous protein secretion, protein digestion in the PJ was sometimes negative. In those cases, a missing value was considered for modelling purposes.
The digestion time (t) was calculated by the estimation of the MRT using the following equation:

$$MRT\ (min.) = (1440 \times C_{mg\ Cr_2O_3/g\ digesta} \times W_{g\ gut}) / I_{mg\ feed\ intake x Cr_2O_3\ in\ feed}$$

where C is the Cr_2O_3 concentration in the digesta, W is the weight of dry gut contents, I is the Cr_2O_3 intake over 24 h and 1440 equals min/d. MRT in the duodenum and in the rectum was not measured and assumed to be 5 and 20 min, respectively (Weurding et al., 2001).
A least-square non-linear iterative process was used to fit the modelling curves of ST and CP disappearance by NLIN procedure of SAS software (SAS Institute, 1985). Data were analysed with ANOVA using the GLM procedure of SAS software (SAS Institute, 1985). The factors were 3 wheat cultivars and 2 levels of N fertilisation. If significant interaction existed between main effects, the data were reanalysed using one-way ANOVA. Means were separated by using Duncan's multiple test. Also, simple Pearson correlation analysis were performed.

RESULTS

The results from the proximate analysis of the six wheat samples used in this experiment are shown in Table 1. Varietal differences in chemical composition were smaller than between crop N fertilisation levels. The differences among cultivars were 4.4% and 12% for ST and CP content, respectively. The increase of crop N fertilisation produced a decrease of 6.2% in ST content but an increase of 38% in CP content. However, viscosity varied more among cultivars (from 4.3 to 9.0 cp) than between N fertilisation (from 5.6 to 7.7 cp). Diets had similar particle size distributions (data not shown) with mean particle size of the experimental diets ranging from 0.66 to 0.70 mm.
Results on ST digestibility in the different segments of the small intestine of broiler chickens are shown in Table 2. Starch was gradually digested along the small intestine, mainly before the ileum (average 84.58% in the jejunum and 96.10% in the PI) with no further digestion after the DI. In the PJ, the ST in Apache cultivar was less digested (43.16%, $P < 0.001$) than ST in the other two cultivars (average 55.80%). The same trend ($P < 0.1$) was observed in the DJ (82.84% vs. average 85.45%). However, no differences ($P > 0.05$) in ST digestibility among wheat cultivars were detected from the PI onwards. No significant effect ($P > 0.05$) of N fertilisation level or wheat-N interaction were found for ST digestion at any of the intestinal segments.

Table 2. Digestion coefficients (%) of starch in different segments of the small intestine of broilers chickens fed diets containing different wheat samples

Effect[1]	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Total Tract
Apache					
N1	44.68	83.43	96.22	97.51	97.96
N2	41.65	82.26	95.71	96.67	97.58
Caphorn					
N1	56.29	82.89	96.58	96.65	98.03
N2	58.14	87.06	96.27	97.11	98.31
Charger					
N1	53.44	85.34	96.24	97.50	98.39
N2	55.31	86.53	95.56	96.45	98.04
Pooled SEM	2.301	1.562	0.695	0.677	0.258
Main effect[2]					
Wheat cultivar					
Apache	43.16[b]	82.84	95.97	97.09	97.77
Caphorn	57.22[a]	84.97	96.42	96.88	98.16
Charger	54.38[a]	85.94	95.91	96.98	98.23
N fertilisation					
N1	51.47	83.89	96.35	97.22	98.14
N2	51.70	85.28	95.85	96.74	97.96
Statistic probability					
Wheat cultivar	<0.0001	0.055	0.720	0.953	0.221
N fertilisation	0.903	0.188	0.384	0.391	0.501
W. cult. x N appl.	0.477	0.121	0.965	0.489	0.423

[a-b] Means within a given column with no common superscript are significantly different (P < 0.05).

[1] Each mean represents 8 experimental units.

[2] Each mean represents 16 and 24 experimental units for wheat cultivar and protein, respectively.

Apparent digestibility of dietary protein increased along the small intestine (from 21.16 to 80.72% in the PJ and DI, respectively), although to a lesser extent than ST digestibility (Table 3). Wheat cultivar affected (P < 0.05) CP digestibility but not consistently. There was

a cultivar-fertilisation interaction ($P < 0.001$) in the PI due to the fact that CP digestibility decreased in Apache cultivar with N fertilisation whereas in the other cultivars CP digestibility increased.

Table 3. Digestion coefficients (%) of protein in different segments of the small intestine of broilers chickens fed diets containing different wheat samples

Effect[1]	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Apache				
N1	22.99	64.41	77.55	80.47
N2	17.62	61.79	75.11	79.01
Caphorn				
N1	18.86	58.27	73.02	79.69
N2	16.62	63.87	77.60	81.56
Charger				
N1	18.38	65.01	76.52	81.31
N2	31.19	66.84	78.74	82.29
Pooled SEM	3.757	1.734	0.876	0.948
Main effect[2]				
Wheat cultivar				
Apache	20.30	63.10^{ab}	76.33^{ab}	79.74^{b}
Caphorn	17.23	61.07^{b}	75.31^{b}	80.62^{ab}
Charger	24.78	65.92^{a}	77.63^{a}	81.80^{a}
N fertilisation				
N1	19.91	62.56	75.69	80.49
N2	22.41	64.16	77.15	80.95
Statistic probability				
Wheat cultivar	0.269	0.026	0.038	0.035
N fertilisation	0.644	0.264	0.048	0.464
W. cult. x N appl.	0.081	0.071	0.0009	0.090

[a-b] Means within a given column with no common superscript are significantly different ($P < 0.05$).

[1] Each mean represents 8 experimental units.

[2] Each mean represents 16 and 24 experimental units for wheat cultivar and N fertilisation level, respectively.

Digestion characteristics of ST and CP, represented by the potential digestibility and the fractional digestion rate, are shown in Table 4. Potential digestibility of ST was 15% greater than the potential digestibility of CP. Fractional digestion rate of ST was almost twice than that of CP. Starch potential digestibility **(Ds)** was very high (average 97.01%), differing ($P = 0.036$) among wheats with Apache showing the greatest Ds (97.78%), followed by Charger (96.98%) and Caphorn (96.34%). However, fractional ST digestion rate (Kds) was ($P < 0.001$) considerably higher in Caphorn (3.28 h^{-1}) than in the other two wheats (average 2.52 h^{-1}). On average, protein potential digestibility (**Dp**) was 84.65% and protein fractional digestion rate (**Kdp**) was 1.53 h^{-1}. Protein digestion characteristics were not affected by wheat cultivar neither by N fertilisation (Table 4). The MRT at jejunum level was different ($P < 0.05$) among wheat cultivars (Table 5). Animals fed Charger showed the greatest MRT in the PJ (14.1 min, $P=0.002$) and in the DJ (41.3 min, $P = 0.009$), followed by the ones fed Caphorn (11.2 and 34.3 min, PJ and DJ respectively) and Apache (9.9 and 33.5 min, PJ and DJ respectively).

AME and AMEn and energy efficiency of the diets were affected both by cultivar and by N fertilisation, although a significant ($P < 0.05$) interaction was observed (Table 6). Caphorn cultivar showed ($P < 0.0001$) about 2% more energy ($P < 0.001$) than the other wheat cultivars. Nitrogen fertilisation negatively affected the energy value of diets containing Charger cultivar (5.7% the AME, 7.2% the AMEn and 2.8% the energy efficiency, $P < 0.05$) whereas no effect was observed on the other wheat cultivars.

DISCUSSION

Total digestion of ST in the 6 wheat samples studied was very high and did not differ among samples. On average, only 2 to 3% of the total ST was undigested at excreta level. These high values of wheat ST digestibilities agreed with our previous research (Gutierrez del Alamo et al., 2008) and with other researchers (Weurding et al., 2001; Carré et al., 2002) and are in concordance with the general assumption that wheat ST is well digested by broiler chickens (Annison, 1990; McNab, 1993; Carré, 2004).

Starch digestion differences among wheat cultivars mainly occurred in the upper part of the small intestine. The Caphorn and Charger wheats showed greater ST digestibility coefficients than Apache wheat. Waldron (1997) performed an experiment where jejunal, ileal and total ST digestibility of two wheat cultivars (Beaver and Dean) were studied. Although variation between individual animals was too high to find significant differences in ST digestion, results suggested that most of the differences for digestion between wheat varieties occurred in the upper part of the small intestine. However, this greater ST digestibility in jejunum does not imply a greater ST digestion rate, as it is important to consider digesta transit time in addition to the digestive capacity in each

Table 4. Starch and protein digestion characteristics in the small intestine of broiler chickens fed diets containing different wheat samples[1]

Effect[2]	Starch		Protein	
	Potential digestibility (Ds, %)	Fractional digestion rate (k_ds), h^{-1}	Potential digestibility (Dp, %)	Fractional digestion rate (k_dp), h^{-1}
Apache				
N1	97.70	2.51	88.15	1.72
N2	97.84	2.40	87.82	1.92
Caphorn				
N1	96.13	3.20	83.42	1.40
N2	96.54	3.35	86.84	1.34
Charger				
N1	97.03	2.71	86.26	1.22
N2	96.92	2.50	85.13	1.55
Pooled SEM	0.527	0.202	1.354	0.229
Main effect[3]				
Wheat cultivar				
Apache	97.78[a]	2.45[b]	83.04	1.83
Caphorn	96.34[b]	3.28[a]	85.13	1.37
Charger	96.98[ab]	2.60[b]	85.70	1.39
N fertilisation				
N1	96.92	2.82	84.28	1.43
N2	97.10	2.75	85.02	1.60
Statistic probability				
Wheat cultivar	0.036	<0.001	0.142	0.10
N fertilisation	0.732	0.742	0.482	0.411
W. cult. x N appl.	0.884	0.654	0.232	0.692

[a-b] Means within a given column with no common superscript are significantly different (P < 0.05).

[1] Starch (and protein) digestion characteristics were calculated using the exponential curve equation DCt = D (1-e$^{-k(d)t}$), where DCt is the proportion of starch (or protein) digested at time t.

[2] Each mean represents 8 experimental units.

[3] Each mean represents 16 and 24 experimental units for wheat cultivar and N fertilisation level, respectively.

segment. In the case of Charger the greater ST digestibility in the jejunum is compensated by a greater MRT producing a ST digestion rate similar to Apache (Table 2, 4 and 5). In fact, a higher and positive correlation was observed for ST digestion rate (r = 0.56) than for ST digestibility (r = 0.21) and dietary AMEn. Rogel et al. (1987) found that total ST digestion among 38 wheat samples ranged between 82 and almost 100% when fed to 6 wk old broiler chickens. However, ST digestion was almost complete when the isolated ST from the 2 samples with the lowest faecal ST digestibility was fed to broiler chickens. This suggests that ST inside the grain is less accessible for the digestive enzymes than when isolated. Starch is located in the endosperm of the cereal grains and it is embedded in a protein matrix (Hoseney, 1998).

McAllister et al. (1993) found that the protein matrix, rather than the chemistry and physical form of the ST, was the major factor responsible for differences in ruminal digestion of ground corn and barley. Therefore, differences in ST digestion among wheat cultivars could depend on their amount of protein. In our study, the N fertilisation produced an increase in CP content of wheat (9.4 vs 13.0%). However, ST digestibility in the PJ differed only among wheat cultivars and not between fertilisation levels. This indicates that the effect of protein as a physical barrier can be different if the protein is genetically determined by the cultivar (i.e. grain hardness, Morris 2002) or it is increased by N fertilization. The fact that the wheat was finely ground may have disrupted the protein matrix opening the ST granules to the enzyme attack and eliminating the problem of accessibility. Due to the fact that particle size distribution of the experimental feeds was not different among diets, differences found among wheat samples cannot be attributed to particle size (Carré, 2000; Svihus and Hetland, 2001).

Analysis of our data showed a positive relationship between MRT and ST digestibility in the PJ (r = 0.44, P = 0.001) and no relationship in the other segments of the small intestine. These results agree with the general knowledge that greater MRT gives to the enzymes more time to act. On the contrary, Weurding et al. (2001) found a negative relationship between MRT and the digestion coefficient of ST and concluded that feedstuffs with low ST digestion coefficient stayed longer in the small intestine.

Table 5. Mean retention time (MRT, min) in the small intestine of broiler chickens fed diets containing different wheat samples

Effect[1]	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Jenunum + ileum
Apache					
N1	10.3	34.9	47.1	52.9	145.2
N2	9.6	32.3	42.5	51.4	135.8
Caphorn					
N1	11.2	34.8	47.3	63.4	156.8
N2	11.2	33.8	48.2	57.5	150.7
Charger					
N1	12.9	43.4	50.1	64.1	170.5
N2	15.3	39.3	46.1	61.0	161.7
Pooled SEM	1.120	2.627	3.886	5.339	10.701
Main effect[2]					
Wheat cultivar					
Apache	9.9[b]	33.5[b]	44.7	52.1	140.1[b]
Caphorn	11.2[b]	34.3[b]	47.8	60.5	153.8[ab]
Charger	14.1[a]	41.3[a]	48.1	62.5	166.1[a]
N fertilisation					
N1	11.5	37.8	48.2	60.4	158.0
N2	12.0	35.1	45.6	56.6	149.4
Statistic probability					
Wheat cultivar	0.002	0.009	0.662	0.142	0.074
N fertilisation	0.555	0.239	0.426	0.434	0.364
W. cult. x N appl.	0.369	0.852	0.740	0.919	0.986

[a-b] Means within a given column with no common superscript are significantly different (P < 0.05).

[1] Each mean represents 8 experimental units.

[2] Each mean represents 16 and 24 experimental units for wheat cultivar and N fertilisation level, respectively.

Table 6. Apparent metabolizable energy (AME, kcal/kg DM) corrected by zero N retention (AMEn) and AMEn efficiency (%) of broiler chickens fed diets containing different wheat samples

Effect[1]	AME	AMEn	AMEn / GE
Apache			
N1	3,453[b]	3,267[bc]	70.5[ab]
N2	3,444[b]	3,252[c]	69.6[bc]
Caphorn			
N1	3,530[a]	3,350[a]	71.2[a]
N2	3,501[a]	3,310[ab]	70.6[ab]
Charger			
N1	3,530[a]	3,353[a]	71.4[a]
N2	3,326[c]	3,109[d]	69.0[c]
Pooled SEM	0.045	0.042	0.002
Main effect[2]			
Wheat cultivar			
Apache	3,448[b]	3,260[b]	70.0[b]
Caphorn	3,515[a]	3,331[a]	70.9[a]
Charger	3,436[b]	3,240[b]	70.3[ab]
N fertilisation			
N1	3503	3324	71.0
N2	3420	3219	69.7
Statistic probability			
Wheat cultivar	< 0.001	< 0.001	0.041
N fertilisation	< 0.001	< 0.001	< 0.001
W. cult. x N appl.	< 0.001	< 0.001	0.022

[a-b] Means within a given column with no common superscript are significantly different (P < 0.05).

[1] Each mean represents 8 experimental units.

[2] Each mean represents 16 and 24 experimental units for wheat cultivar and N fertilisation level, respectively.

Results of our trial regarding apparent CP digestibility and CP digestion characteristics should be interpreted with care because of the presence of variable amounts of endogenous protein in the digesta. This was especially observed in the PJ where negative CP digestibility coefficients were

found probably due to the balance between endogenous production and digestion. According to Golian et al. (2008) the ileal endogenous amino acid flow represents about 0.44% of the DM intake. If our ileal CP digestibility data (average 81%) were corrected by this contribution of endogenous protein, they increase to 92%, values similar to that obtained by Siriwan et al. (1993). Although in some intestinal segments CP digestibility coefficients differed among wheat cultivars and N fertilisation, the curves that described the CP disappearance are similar. This seems to indicate that dietary CP digestion rate itself is not affected by wheat cultivar or N fertilisation.

In the literature there is high correlation between ST digestibility and AME value of wheat (Rogel et al., 1987; Wiseman et al., 2000). However, if ST digestibility is at a high level, its variation does not always relate to variation in AMEn (Gutierrez del Alamo et al., 2008). In our study, AMEn correlated with ST digestibility in the PI ($r = 0.40$, $P < 0.05$). This indicates that assessment of either excreta or ileal digesta is not the most accurate means to measure ST digestibility, especially if ST digestibility is almost complete at the end of the ileum.

Weurding (2002) observed that broiler chickens performed better when considerable amount of ST was digested after the PI. Based on that observation, he concluded that slowly digestible ST improves broiler performance. In our study, the majority of the ST was digested before or in the PI. Hence, no effect on broiler growth should be expected. However, Waldron (1997) found differences in broiler growth when two wheat varieties (Beaver and Dean) were fed to broiler chickens from 7 to 21 d. In both varieties, the majority of the ST was already digested by the time the digesta reached the DI.

In conclusion, almost all the ST contained in the wheat grain is digested by the time the digesta reaches the DI. The rate of ST digestion is affected by wheat cultivar but not by N fertilisation level. On the contrary, dietary CP digestion rate is not affected by wheat cultivar or N fertilisation level. The digestion rate of ST is faster than that of CP. The current study suggests that wheat cultivars can be classified based in their rate of ST digestion independently on the N fertilisation applied to the crop during growth. The effect of the observed difference in rate of ST digestion among wheat cultivars and the different digestion rate between ST and CP on animal performance warrant further investigation.

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CHAPTER 6

Wheat Starch Digestion Rate Affects Broiler Performance

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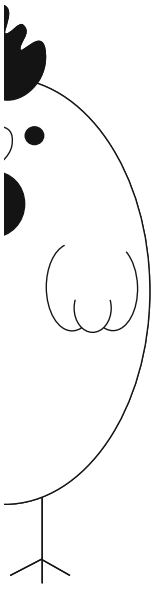

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ABSTRACT Two experiments were conducted to determine the differences in starch (**ST**) digestion rate **(KDS)** among wheats from different cultivar and origin and to verify if chickens would benefit from a certain digestion rate of starch. In the first experiment, 192 chickens (21 d) were assigned to 4 diets containing 55% of each wheat sample (3 cultivars, one of them from 2 origins). Starch and protein digestion were calculated from the remaining ST and protein in four segments of the small intestine and in excreta, using chromic oxide as a marker. Mean retention time was measured in each segment which enable calculations of digestion rates. In the second experiment, 2,600 chickens were assigned to 5 isoenergetic and isonitrogenous diets (with KDS from 1.80 to 2.56 h^{-1}) and growth performance was determined (1 to 34 d). In 3 treatments dietary ST was provided each by the wheat cultivars (same origin) whereas in the other 2 treatments, 25 and 50% of the wheat starch with the highest KDS was substituted by pea ST. *Clostridium perfringens* and *Lactobacillus* in the cecal chyme and glucose in the blood (glycemic index) were measured in broilers at d 19 and d 25, respectively. Starch was gradually digested along the small intestine, mainly in the upper part (48.5 and 80.4% at proximal and distal jejunum) where the largest differences among wheat samples were found. Starch digestion rate varied with origin (from 1.96 to 2.56 h^{-1}) and cultivar (from 2.17 to 2.56 h^{-1}). Crude protein digestion rate (average 2.21 h^{-1}) was not affected by either cultivar or origin. Broiler growth and feed conversion ratio improved in a linear and quadratic way with KDS. The maximum broiler performance was observed with KDS around 2.2 h^{-1}. Blood glucose response (glycemic index) was not affected by KDS, therefore it cannot be used to predict animal performance. It was concluded that KDS varies among wheat cultivar and origin and its variation affects animal performance.

(Key words: wheat, starch digestion rate, broiler performance, glycemic index, microflora counts)

INTRODUCTION

Wheat is a common raw material in European broiler diets because of its high availability, good ratio price:quality and its lack of pigments. Therefore, it is relatively common to find wheat as the only cereal in broiler diets with inclusion percentages of 55% where it contributes up to 60-65% of the AMEn and 35-40% of the crude protein **(CP)** of the diet. When a feedstuff accounts for a major proportion of the dietary nutritional value, a precise knowledge of its digestion characteristics and utilization is of extreme importance. In this sense, many studies about the use of wheat grain in poultry nutrition have been developed, revealing a great variability on wheat utilization by chickens among samples. For example, Mollah et al. (1983) found wheat AMEn ranging between 2.63 and 3.80 Mcal/kg DM (n = 22) and Scott et al. (1998) observed a wide variation (13%) in broiler performance produced by different wheat samples. Additionally, recent studies have evidenced a low or no relationship between wheat AMEn and broiler performance (Scott 2000, Steendfeldt 2001, Gutierrez del Alamo et al. 2008a). Therefore, new ways of explaining the interaction between wheat and broiler performance should be explored.

As starch **(ST)** is the major energy yielding component inside cereals, attention should be paid to its digestion. Although fecal ST digestibility of wheat grain is almost total, differences in ST digestion rate **(KDS)** among wheat cultivars have been found (Gutierrez del Alamo et al., 2008b). However, it is still not known if different growing conditions of wheat cultivars affect KDS in a similar way as their physico-chemical properties (McCracken et al., 2002; Pirgozliev et al., 2003) or if the differences observed in KDS among wheats affect broiler performance. Weurding (2002) showed that broilers grew more efficiently with low dietary KDS values (closer to 1.26 h^{-1}). The positive effect of the slowly digestible ST rate on chicken performance was suggested to result from a better synchronization of energy to protein availability and to a more continuous supply of glucose to the intestinal lumen. The rate of ST digestion could be reflected in plasma glucose levels and glycemic index (Englyst et al., 1996). Moreover, the amount of ST that arrives at the large intestine modifies the microflora profile (Kleesen et al., 1997). Therefore, *Clostridium perfringens* could be controlled by modifying dietary KDS.

The objectives of the present study were to determine i) the effect of cultivar and growing location (origin) on ST digestion rate and, ii) the effect of diets with different ST digestion rate mainly coming from wheat on broiler performance, glycemic index and Clostridium perfringens and Lactobacillus counts in cecal chyme.

MATERIAL AND METHODS

All the procedures described in the present experiments were performed following the principles for care of animals in experimentation (Spanish Royal Decree 1201/2005, 2005).

Experiment 1

Wheat Cultivars and Feeds. A total of four wheat samples (Astral, Isengrain, Marius1 and Marius2) were used in this experiment (Table 1). The samples Astral, Isengrain and Marius1 were grown in the same field of El Viso de San Juan (Toledo, Spain) whereas the sample Marius2 was grown in Ayllón (Segovia, Spain). Each wheat sample was incorporated in diets which met or exceeded broiler requirements (NRC, 1994) (Table 2). Chromic oxide **(Cr_2O_3)** was included (0.5%) as an indigestible marker. Wheats were milled separately and passed through a 2.5-mm screen in a hammer mill (Rosal, 40 horse power, Barcelona, Spain) and then mixed with the other ingredients to constitute the experimental diets. Diets were presented as a mash and offered for ad libitum consumption to the chickens.

Table 1. Chemical and physical properties of the different wheat cultivars

Parameter[1] (% DM)	Astral	Isengrain	Marius1	Marius2
Dry matter (%)	90.10	89.45	89.87	90.97
Starch	64.59	67.76	63.79	63.98
Crude protein	13.14	14.80	12.70	16.29
Crude fiber	2.77	2.52	2.45	2.69
Ether extract	1.78	1.76	1.65	1.65
Ash	2.19	2.21	2.18	1.59
NDF	15.76	14.81	14.80	15.72
ADF	3.27	2.52	3.12	3.13
ADL	1.33	1.01	1.11	1.04
Gross energy (kcal/kg DM)	4,376	4,397	4,371	4,484
Viscosity (cp)	3.81	3.35	3.13	5.98
Specific weight (kg/hl)	73.00	76.00	75.00	75.90

[1] *NDF = neutral-detergent fiber; ADF = acid-detergent fiber; ADL = acid-detergent lignin*

Animals and Experimental Design. In an in vivo experiment, KDS and CP digestion rate **(KDCP)** of the 4 experimental diets were analyzed. Starch in wheat was the only ST source in the experimental diets. Crude protein was provided by wheat, soya bean and by synthetic amino acids, although the variation among diets is only attributed to wheat samples.

Table 2. Ingredient profile and determine analysis of diet used in experiment 1

Item	
Ingredient (%)	
Wheat	55.00
Full-fat soybean	27.00
Soybean meal	11.34
Soybean oil	2.38
Salt	0.30
Limestone	2.00
Monocalcium phosphate	1.10
Sodium bicarbonate	0.18
L-Lys	0.18
DL-Met	0.22
Mineral-vitamin premix	0.30[1]
Determined analysis[2] (%)	
Dry matter	89.44
Crude protein	22.15
Starch	34.05
Crude fiber	3.31
Ash	6.86
Gross energy (kcal/kg DM)	4,693

[1] Supplied per kg diet: cobalt , 0.2 mg; copper, 8 mg; iron, 20 mg; manganese, 80 mg; zinc, 59 mg; selenium, 0.2 mg; iodine, 2 mg; vitamin A, 9,999 IU; vitamin E, 29 IU; cholecalciferol, 50 µg; vitamin B1, 2 mg; vitamin B2, 8 mg; vitamin B6, 1.5 mg; vitamin B12, 0.01 mg; vitamin K, 3 mg; pantothenate, 10 mg; folic acid, 1 mg; niacin, 50 mg; biotin, 0.1 mg; choline, 250 mg; B.H.A plus ethoxyquine, 100 mg; salinomycin, 70 mg.

[°] Mean values of the 4 experimental diets.

A total of 300 one-day old male broiler chickens (Ross 308) were obtained from a commercial hatchery (Cazalegas, Toledo, Spain). Animals were fed a mash standard diet covering nutrient requirements (NRC, 1994) until d 21. At d 21, chickens were weighed individually and 192 of them with similar body weight (BW 740 ± 51 g) were selected for the experiment. Chickens were assigned individually to battery cages (19.5 x 38.5 x 35 cm, width x length x height) at random. Six cages formed one replicate and each experimental diet was supplied to 8 replicates. Ambient temperature and light in the house were 22ºC and 16 h light per day, respectively. Feed intake was monitored from d 21 until the end of the experiment at d 30. The average FI of the last 3 days of the experimental period (d 27 to d 30) was used to calculate the mean retention time **(MRT)**. Excreta were collected per replicate for 3 consecutive days, from d 27 to d 30. Contaminations, such as down and feathers, were carefully removed and the excreta was stored in containers at –20ºC. Afterward, excreta was dried at 70 ± 0.5ºC for 48 hours. Samples collected from each replicate (6 chickens) during the 3 d of excreta collection were blended, ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored at 4ºC in plastic cans until analysis.
At the end of the experiment (d 30) all birds were euthanized by cervical dislocation. Immediately afterward the small intestine was removed, the mesenterium was cut, and jejunum and ileum were separated at Meckel's diverticulum. Jejunum and ileum contents were collected as described by Weurding et al. (2001). In this procedure, both jejunum and ileum were each split into two parts of equal length (proximal jejunum, **PJ**; distal jejunum, **DJ**; proximal ileum, **PI** and distal ileum, **DI**) and digesta were rinsed out of each segment (without squeezing) by using demineralized water at 4ºC. Digesta were collected separately per segment (PJ, DJ, PI and DI) and replicate (6 chickens) and stored at –80ºC until they were freeze-dried. After freeze-drying, the samples were ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored at 4ºC in plastic bottles until analysis.

Experiment 2

Wheat Cultivars and Feeds. In this experiment the effect of the rate of ST digestion on animal performance was studied. The three wheat cultivars (Astral, Isengrain and Marius1) grown in the same region and studied in the first experiment were used in the current one. As the range of dietary KDS achieved by the solely inclusion of wheat was narrow, two other diets were formulated where 25 and 50% of starch from Marius1 (2.56 h^{-1}) was replaced by peas ST (with a presumably much lower KDS, 1.03 h^{-1}, Weurding et al., 2001) (Table 3). In this way the first diet would have a theoretical KDS similar than that of Isengrain diet (2.17 h^{-1}) that would confirm the KDS assigned to peas. The second diet would increase the range of KDS. Therefore, the different KDS levels studied were 1.80 h^{-1} (Marius1 50% and peas 50%), 2.17 h^{-1} (Isengrain), 2.20 h^{-1} (Marius1 75% and peas 25%), 2.48 h^{-1} (Astral) and 2.56 h^{-1} (Marius1).

Table 3. Ingredient profile and determine analysis of starter- grower- and finisher diets fed to broiler chickens in experiment 2

	Starter (d 0 to d 21)					Grower (d 21 to d 34)				
Item[1]	Marius1-P50	Isengrain	Marius1-P25	Astral	Marius1	Marius1-P50	Isengrain	Marius1-P25	Astral	Marius1
KDS[2] (h^{-1})	1.80	2.17	2.20	2.48	2.56	1.80	2.17	2.20	2.48	2.56
Ingredient (%)										
Wheat	28.00	55.00	43.00	55.00	55.00	28.00	55.00	43.00	55.00	55.00
Peas	35.00	-	17.00	-	-	35.00	-	17.00	-	-
Full-fat soybeans, toasted	27.00	27.00	27.00	27.00	27.00	25.00	25.00	25.00	25.00	25.00
Soybean meal	2.30	11.20	6.15	11.20	11.20	3.41	12.66	7.46	12.66	12.66
Soybean oil	3.20	1.78	2.22	1.78	1.78	4.29	2.84	3.31	2.84	2.84
Salt	0.27	0.21	0.23	0.21	0.21	0.25	0.26	0.25	0.26	0.26
Limestone	1.50	1.87	1.50	1.87	1.87	1.35	1.52	1.27	1.52	1.52
Monocalcium phosphate	1.65	1.66	1.66	1.66	1.66	1.75	1.76	1.76	1.76	1.76
Sodium bicarbonate	0.37	0.45	0.43	0.45	0.45	0.36	0.35	0.35	0.35	0.35
L-Lys	0.05	0.22	0.16	0.22	0.22	-	0.10	0.04	0.10	0.10
DL-Met	0.33	0.26	0.30	0.26	0.26	0.29	0.21	0.26	0.21	0.21
L-Thr	0.03	0.05	0.05	0.05	0.05	-	-	-	-	-
Mineral-vitamin premix	0.30[3]	0.30[3]	0.30[3]	0.30[3]	0.30[3]	0.30[4]	0.30[4]	0.30[4]	0.30[4]	0.30[4]
Determined analysis (%)										
DM	89.94	90.36	90.06	90.32	90.22	91.09	91.02	91.07	91.03	91.06
CP	22.05	22.17	21.32	22.08	21.94	20.68	22.05	20.98	22.01	21.68
Ether extract	7.95	6.80	7.35	6.80	6.35	9.00	8.05	8.25	8.25	8.00
Starch	35.66	35.88	35.77	34.73	36.04	30.55	29.85	31.85	29.30	31.55
Crude Fiber	4.28	3.59	3.75	3.89	3.66	4.59	3.65	3.96	4.01	3.83
AMEn (kcal/kg)[5]	2,850	2,836	2,850	2,850	2,851	2,900	2,886	2,900	2,900	2,900

[1] Dietary total starch provided by the different cultivars; Marius1-P25, Marius1-P50 = diet containing peas at inclusion percentage that supplied 25% and 50% of total dietary starch, respectively. [2] KDS of Marius1-P25 and Marius1-P50 calculated with a KDS for peas of 1.03 h^{-1} (Weurding et al., 2001). [3] Supplied per kg of diet: cobalt , 0.2 mg; copper, 12 mg; iron, 20 mg; manganese, 90 mg; zinc, 59 mg; selenium, 0.2 mg; iodine, 2 mg; vitamin A, 9,999 IU; vitamin E, 37 IU; cholecalciferol, 75 µg; vitamin B_1, 2 mg; vitamin B_2, 12 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.015 mg; vitamin K, 4 mg; pantothenate, 12 mg; folic acid, 1.5 mg; niacin, 50 mg; biotin, 0.15 mg; choline, 350 mg; B.H.A plus ethoxyquine, 100 mg; narasin, 8 mg; nicarbacin, 8 mg. [4] Supplied per kg of diet: cobalt , 0.2 mg; copper, 8 mg; iron, 20 mg; manganese, 80 mg; zinc, 59 mg; selenium, 0.2 mg; iodine, 2 mg; vitamin A, 9,999 IU; vitamin E, 29 IU; cholecalciferol, 50 µg; vitamin B_1, 2 mg; vitamin B_2, 8 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 0.01 mg; vitamin K, 3 mg; pantothenate, 10 mg; folic acid, 1 mg; niacin, 50 mg; biotin, 0.1 mg; choline, 250 mg; B.H.A plus ethoxyquine, 100 mg; salinomycin, 70 mg. [5] Calculated by CVB, 2007.

For each growth period (starter and grower) five dietary compositions were formulated to be isoenergetic and isonitrogenous and with similar ST content. All diets were formulated to meet or exceed broiler chicken nutrient requirements (NRC, 1994) (Table 3). Diets were presented as mash and offered ad libitum to the chickens.

Animals and Experimental Design. A total of 2,600 one-day old male broiler chickens (Ross 308) were obtained from a commercial hatchery (Cazalegas, Toledo, Spain) and assigned randomly to 40 pens (7.5 m^2), each pen contained 65 broiler chickens. Each experimental diet was assigned randomly to 8 pens. Each pen was equipped with one hanging feeder, a nipple drinker and wood shavings (6 cm-deep) as litter. Feed and water were provided for ad libitum consumption during the whole experiment. Light was on continuously during the first day and during 16 hours per day afterwards. Broiler chickens were kept at 32ºC during the first wk of age. Thereafter temperatures were decreased with 3ºC per wk to reach 22ºC by 23 d of age. After that, they were maintained around 22ºC until the end of the experiment.
The experimental period lasted 34 d, animals were weighed at the beginning (d 0), when the experimental diets changed from starter to grower (d 21) and at the end, prior to slaughtering (d 34). The chickens were inspected daily and the dead birds were collected and the date and their body weight were recorded. To calculate FCR, the BW of dead birds was considered also.
Clostridium perfringens and Lactobacillus determination. One animal per pen was randomly selected on d 19 and euthanized. Immediately afterward the caeca were removed and the content was collected by gentle finger-stripping. Caeca content was introduced into sterile polystyrene tubes and stored at room temperature (16ºC) in anaerobiosys bags GENbag (Bio-Mérieux S.A., Marcy létoile, France) to keep the microflora viability. Clostridium perfringens and Lactobacillus were placed under incubation within 6 h after collection. Clostridium perfringens was analyzed according to the method ISO 7937 (1997). Lactobacillus were counted on MRS agar (Biokar Diagnostics, France) after incubation in an anaerobic chamber at 37ºC for 48 h.
Blood glucose response and glycemic index determination. Additionally, another 195 one-day old male broiler chickens were housed in the same room as in experiment 2 and fed on a mash starter diet for the first 16 d. At d 17, all birds were weighed, sixty were selected (BW 423 ± 66 g) and moved to another room prepared with metabolic cages (19.5 x 38.5 x 35 cm, width x length x height). Each cage was fitted with a metal feeder and a drinker. Blood glucose was measured in vivo at d 25 following a modified version of the method described by Denardin et al. (2007). For that, after 7 d of adaptation to the cages and diets (from d 18 to d 24), chickens were fasted 12 h overnight. The following day (d 25) chickens were tube-fed

either 7 g of glucose or 20 g of each of the experimental diets that contained approximately 7 g of available carbohydrates. In total 10 broilers were assigned to each feed. Blood samples of wing vein were taken for glucose levels at fasting (before the tube-feeding) and 30, 50, 70, 130, 240 and 420 min after the meal and glycemic index was calculated.

Chemical Analysis

All analysis, except Cr_2O_3 in feed (8 times/treatment), were carried out in duplicate and the results were expressed on a DM basis. Chemical analysis of the wheats, diets and excreta were conducted according to the methods of AOAC International (1995) for DM (930.15), N (954.01), crude fiber (962.09), ether extract (960.39) and ash (942.05). Neutral-detergent fiber, acid-detergent fiber and acid-detergent lignin were determined following the procedures described by Van Soest et al. (1991). Starch content in experiment 1 was analyzed following the alpha-amylogluclosidase method (996.11, AOAC International, 1995). Starch content of experimental diets in experiment 2 was analyzed polarimetrically (Spanish Royal Decree 2257/1994, 1994). Viscosity of the grains was measured according to Bedford and Classen (1993). Chromium oxide content in feed and excreta was determined according to Fenton and Fenton (1979). Gross energy values were determined by bomb calorimeter using Parr 6100 adiabatic calorimeter (Parr Instrument Company, Moline, IL, USA). Particle size of diets was determined by dry sieving to determine particle size distribution (Pfost and Headley, 1976). Blood glucose of the chickens was determined using Accu-Chek Active® (Roche, Switzerland) monitoring kit after being validated by a previous assay where a 3% repeatability and a 0.99 linearity between 50 and 550 mg/dl was obtained.

Calculations and Statistical Analysis

Apparent digestibility coefficients of ST and CP were calculated in each intestinal segment (PJ, DJ, PI, DI) and in the total tract by calculating the ratio Cr_2O_3 concentration in the feed and digesta or excreta.

The digestion time (t) was calculated by the sum of the different MRT determined in each intestinal segment. Mean retention time was calculated using the following equation:

$$MRT\ (min) = (1440 \times C_{\text{mg } Cr_2O_3/\text{g digesta}} \times W_{\text{g gut}}) / I_{\text{mg feed intakex } Cr_2O_3 \text{ in feed}}$$

where C is the Cr_2O_3 concentration in the digesta, W is the weight of dry gut content, I is the Cr_2O_3 intake over 24 h and 1440 equals min/d. Mean retention time in the duodenum and in the cloaca was not measured and assumed to be 5 and 20 min, respectively (Weurding et al., 2001).

By relating the digestion coefficient achieved in each segment with the digestion time (t), the pattern of fractional disappearance rate was studied. In the case of ST the equation developed by

Ørskov and McDonald (1979) was used:

$$DS = DST \times (1-e^{-KDS \times t})$$

where DS is the percentage of ST that disappeared at time t and the fraction DST is the amount of potential digestible ST (asymptote) which is digested at a fractional rate KDS (per unit time, h^{-1}). Therefore, a KDS of 1 would mean a 100% ST disappearance within an hour. Starch absorption was assumed not to take place prior to the small intestine. In the case of CP fractional disappearance rate the presence of endogenous protein in the small intestine produced negative values in the PJ. Therefore, a modification of the model previously described was used (Denham et al., 1989):

$$DP = DCP \times (1-e^{-KDCP \times (t-t0)}) \; t > t_0$$

where DP is the percentage of CP that disappeared at time t, the fraction DCP is the amount of potential digestible CP (asymptote) which is digested at a fractional rate KDCP (per unit time, h^{-1}), and t0 represents the time in which CP digestion equals to endogenous protein secretion. When digestion time (t) was below t_0, DP was assumed to be 0.

Glycemic index for each experimental diet was calculated by the ratio between the areas under the curves plotted with the blood glucose responses of chickens fed the experimental diets or the pure glucose (reference food) (Frost et al., 1993).

A least-square non-linear iterative process was used to fit the modeling curves of ST and CP disappearance by NLIN procedure of SAS software (SAS Institute, 1985). Data from experiment 1 were analyzed by non-orthogonal contrasts evaluated by Bonferroni t statistics. Linear and quadratic effects on broiler chickens performance (experiment 2) were tested with five levels of KDS using the ProcReg statement of SAS software (SAS Institute, 1985). Blood glucose response was analyzed by using 1-way ANOVA repeated measurements. To obtain normal distribution for statistical analysis mortality data were transformed into arcsine of the square root and microbial colony-forming unit was transformed into logarithm (log10).

RESULTS

The results from the proximate analysis of the four wheat samples used in this experiment are shown in Table 1. Largest differences in ST content were observed between Marius1 and Isengrain (63.79 vs. 67.76%) whereas Astral and Marius2 showed intermediate ST content (average 64.28%). Crude protein content varied from 12.70 to 16.29% for the same cultivar from different origin. All wheat samples showed a specific weight above 72 kg/hl.

Experiment 1

Experimental diets had similar particle size distributions and their mean particle size ranged from 0.69 to 0.71 mm (data not shown).

Starch digestion characteristics are shown in Table 4. Starch was gradually digested along the small intestine, mainly before the DI (80.43% digested in the jejunum and 93.99% was digested already in the PI) with hardly any ST digestion after the DI (1.5% difference between DI and total tract). Largest differences among wheat cultivars were observed in the PJ whereas the effect of origin was most pronounced in the DJ. Differences observed in total tract ST digestibility were due to differences in the jejunum as no differences in the ileum occurred. Marius1 showed numerically greater ($P = 0.052$) total tract ST digestibility than Marius2 (97.27 vs. 96.19%) whereas a cultivar effect was observed between animals fed Astral and Isengrain (98.13 vs. 96.34%, $P = 0.003$, respectively).

An example of the pattern of ST disappearance along the small intestine related to its digestion time and how it fits with the mathematical model used is shown in Figure 1. Potential ST digestibility was an average of 96.08% (Table 4) with no differences ($P = 0.93$) exhibited among wheat samples. Fractional digestion rate of ST was affected by both origin and cultivar. Animals fed Marius1 showed greater ($P = 0.001$) KDS than those fed Marius2 (2.56 vs. 1.96 h^{-1}, respectively). There were differences in KDS among animals fed wheat cultivar Isengrain compared to Marius1 (2.17 vs. 2.56 h^{-1}, $P = 0.028$, respectively).

Table 4. Digestion coefficients of starch and starch digestion characteristics in the small intestine segments of broiler chickens fed diets containing different wheat samples[1]

Item[2] (%)	Astral (A)	Isengrain (I)	Marius1 (M1)	Marius2 (M2)	Pooled SEM	Contrast			
						Cultivar A vs. I	Cultivar A vs. M1I	Cultivar vs. M1	Origin M1 vs. M2
Jejunum									
Proximal	56.05	47.74	48.20	41.92	2.88	0.051	0.064	0.910	0.134
Distal	80.02	81.15	83.20	77.38	1.41	0.576	0.123	0.314	0.007
Ileum									
Proximal	95.22	93.11	94.78	92.86	0.94	0.127	0.744	0.224	0.162
Distal	95.21	95.47	96.10	95.15	1.07	0.865	0.562	0.680	0.538
Total tract	98.13	96.34	97.27	96.19	0.36	0.003	0.132	0.101	0.052
Potential starch digestibility (DST)	96.66	95.54	96.02	96.11	0.85	0.995	0.686	0.690	0.936
Fractional starch digestion rate (KDS) (h^{-1})	2.48	2.17	2.56	1.96	0.13	0.077	0.638	0.028	0.001

[1] Starch digestion characteristics were calculated using the exponential curve equation DS = DST x (1-e-^{KDSt}), where DS is the proportion of starch digested at time t and the fraction DST is the amount of potential digestible starch (asymptote) which is digested at a fractional rate KDS (per unit time, h^{-1}).

[2] Each mean represents 8 experimental units of 6 broiler chickens each.

Table 5. Digestion coefficients of protein and protein digestion characteristics in the small intestine segments of broiler chickens fed diets containing different wheat samples[1]

Item[2] (%)	Astral (A)	Isengrain (I)	Marius1 (M1)	Marius2 (M2)	Contrast				
					Pooled SEM	Cultivar A vs. I	Cultivar A vs. M1I	Cultivar vs. M1	Origin M1 vs. M2
Jejunum									
Proximal	22.68	26.10	11.66	26.29	4.74	0.614	0.112	0.040	0.044
Distal	62.92	67.28	63.51	63.24	1.77	0.092	0.814	0.143	0.913
Ileum									
Proximal	76.84	78.19	76.24	76.60	1.06	0.377	0.693	0.205	0.814
Distal	78.81	79.98	80.35	81.48	5.19	0.611	0.502	0.869	0.624
Total tract[3]	59.91	59.02	59.60	57.29	0.97	0.551	0.832	0.699	0.118
Potential protein digestibility (DCP)	79.81	80.37	79.93	81.58	1.32	0.775	0.949	0.824	0.524
Fractional protein digestion rate (KDCP) (h^{-1})	2.09	2.18	2.43	2.17	0.16	0.707	0.156	0.290	0.295
t_0[4]	9.27	7.03	11.46	8.23	2.05	0.448	0.457	0.132	0.291

[1] Protein digestion characteristics were calculated using the exponential curve equation $DP = DCP\ x\ (1\text{-}e^{-KDCP\ x\ (t\text{-}t0)})$*, where DP is the proportion of protein digested at time t, the fraction DCP is the amount of potential digestible crude protein (asymptote) which is digested at a fractional rate KDCP (per unit time,* h^{-1}*), and t0 represents the time in which crude protein digestion equals to endogenous protein secretion.*

[2] Each mean represents 8 experimental units of 6 broiler chickens each.

[3] Values reported refers to CP retention.

[4] Time in which crude protein digestion equals to endogenous protein secretion.

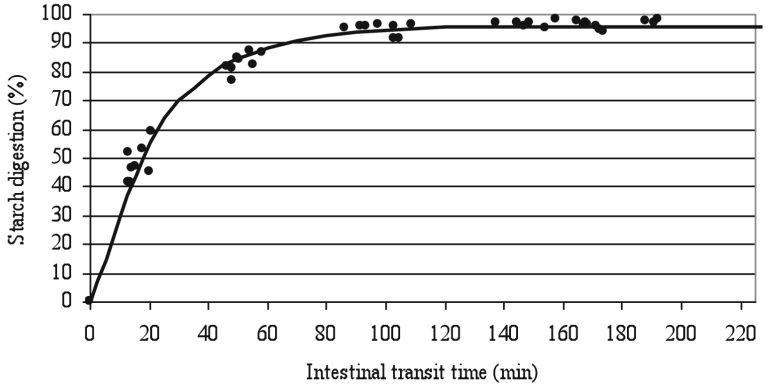
Table 6. Mean retention time (MRT, min) in the small intestine of broiler chickens fed diets containing different wheat samples

Item[1] (%)	Astral (A)	Isengrain (I)	Marius1 (M1)	Marius2 (M2)	Pooled SEM	Contrast			
						Cultivar A vs. I	Cultivar A vs. M1I	Cultivar vs. M1	Origin M1 vs. M2
Jejunum									
Proximal	15.19	13.57	10.97	12.88	1.46	0.440	0.051	0.220	0.365
Distal	35.18	39.72	35.44	36.59	2.05	0.130	0.930	0.152	0.695
Ileum									
Proximal	51.82	51.32	47.12	46.23	2.47	0.888	0.191	0.240	0.801
Distal	55.15	73.13	56.93	57.91	9.36	0.185	0.894	0.231	0.941
Jejunum + Ileum	157.35	177.76	150.48	153.62	9.78	0.151	0.623	0.058	0.821

[1] *Each mean represents 8 experimental units of 6 broiler chickens each..*

Digestion characteristics of dietary CP are shown in Table 5. Dietary CP was gradually digested along the small intestine, mainly before the DI (average 64.23% in the jejunum and up to 76.96% at the end of the ileum). Between the PI and the DI an increase of 4% in dietary CP digestibility was observed (from 76.96 to 80.15%). The calculation of the time in which CP digestion was inferior to endogenous protein secretion (t_0) did not differ among wheat samples (average 8.99 min). Although some effects of cultivar and origin were observed in CP digestion in PI or DI, these effects did not appear in the ileum. As a consequence, fractional CP digestion rate was not affected by wheat sample, being as average 2.21 h^{-1}.
Total MRT of diets in the jejunum plus ileum was as an average 159.80 min (Table 6). Mean retention time in the jejunum was 13.15 and 36.73 min for the PJ and the DJ, respectively, whereas in the ileum the MRT was 49.12 and 60.78 min for the PI and the DI, respectively. No effect of wheat origin was observed.

Figure 1. Starch digestion along the intestine for wheat Marius1. Each point represents the rate of starch digestion of a replicate (6 chickens)



Experiment 2.
Rate of ST digestion of experimental diets had a quadratic effect on most broiler growth variables (Table 7). At d 21, daily gain **(DG)** and FCR improved quadratically ($R^2 = 0.52$ and 0.56, respectively, $P < 0.001$) with KDS. The best DG (32.44 g/d) and FCR (1.383) were obtained for chickens fed a diet with KDS 2.17 h^{-1}. However, daily feed intake **(DFI)** (average 44.90 g/d) was not affected by KDS. During the grower period also a quadratic response was observed for DG ($R^2 = 0.47$, $P = 0.008$), DFI ($R^2 = 0.21$, $P = 0.008$) and FCR ($R^2 = 0.57$, $P < 0.001$). This quadratic response was more pronounced in the overall period for DG and FCR

(R^2 = 0.62 and 0.73, respectively, $P < 0.001$) where the best results (53.39 g/d and 1.572, respectively) were obtained again for KDS 2.17 h^{-1}. Dietary treatments did not affect mortality (average 6.7%) in any of the periods studied.

Blood glucose changed with time after feeding ($P < 0.0001$), going up to 253 mg/dl within 70 min after feeding, and thereafter gradually decreasing until 212 mg/dl at 420 min (Figure 2). There was no effect of dietary treatments on blood glucose levels ($P = 0.707$). An interaction between time and diet for glucose level was observed ($P = 0.022$). From 50 to 130 min a decrease in blood glucose response (from 259 to 239 mg/dl) was noted when chickens were fed diets including peas, whereas those which received only ST from wheat maintained or even increased their blood glucose level. Glycemic index compared to glucose (100%) ranged between 60 and 65% (data not shown).

No differences in cecal counts of Clostridium perfringens ($P = 0.839$) and Lactobacillus ($P = 0.369$) among diets were observed being as average 4.26 and 8.96 log cfu, respectively (Figure 3).

DISCUSSION

Starch was gradually digested along the small intestine with less than 4% undigested ST entering the large intestine. Most of the differences among wheat samples occurred in the jejunum and were responsible for the different KDS values observed. The importance of the upper part of the small intestine (jejunum) in wheat ST digestion has been found in a previous work where ST digestion was affected by wheat cultivar but not by crop nitrogen fertilization (Gutierrez del Alamo et al., 2008b). In the current work we found differences in ST digestion of the same wheat cultivar when grown at two different locations, despite their chemical composition mainly varied in CP (from 12.70 to 16.29%) as in the previous trial. This seems to indicate that besides cultivar, other factors different to crop nitrogen fertilization but related to origin (i.e. soil, environmental conditions) produces physico-chemical changes inside the wheat grain (George and McCracken, 2003; Kim et al., 2003; Pirgozliev et al., 2003) and modifies its digestion by broiler chickens. Therefore, variety of wheat alone does not reflect the KDS of wheat by broiler chickens. Among wheat chemical composition, CP seemed to have a great negative correlation to KDS, although only 4 wheat samples were evaluated. When samples of the previous study (Gutierrez del Alamo et al., 2008b) were combined with this one and analyzed together, this correlation decreased, indicating a different effect of genetically or environmental defined CP.

Table 7. Effect of diets containing different levels of rate of starch digestion on broiler performance (mean values)

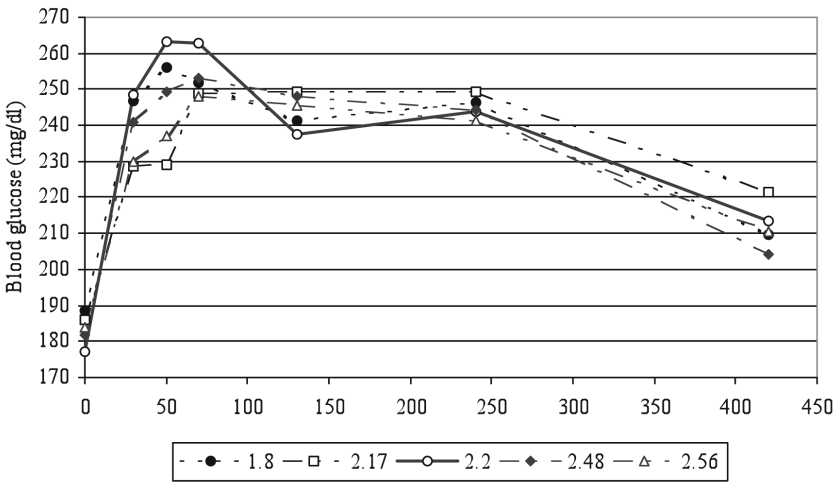
Item[1,2,3]	Marius1-P50	Isengrain	Marius1-P25	Astral	Marius1	Pooled	Analysis	
KDS[4] (h^{-1})	1.80	2.17	2.20	2.48	2.56	SEM	Linear	Quadratic
Starter period (d 1 to d 21)								
BW at 21 d, g	675	722	717	711	698	6.11	<0.0001	<0.0001
DG, g/d	30.22	32.44	32.22	31.93	31.32	0.29	<0.0001	<0.0001
DFI, g/d	44.58	44.88	45.69	45.07	44.27	0.41	0.125	0.116
FCR	1.484	1.383	1.418	1.411	1.413	0.01	<0.0001	<0.0001
Mortality (%)	6.92	6.15	4.39	6.34	7.32	1.09	0.109	0.106
Grower period (d 21 to d 34)								
BW at 34 d, g	1,744	1,856	1,837	1,826	1,833	10.88	<0.0001	<0.0001
DG, g/d	82.22	87.22	86.17	85.79	87.30	0.67	0.004	0.008
DFI, g/d	157.44	153.88	149.72	152.91	155.71	1.59	0.007	0.008
FCR	1.915	1.764	1.738	1.782	1.784	0.02	<0.0001	<0.0001
Mortality (%)	0.64	0.68	0.23	0.40	0.41	0.35	0.810	0.830
Overall period (d 1 to d 34)								
DG, g/d	50.10	53.39	52.85	52.52	52.72	0.31	<0.0001	<0.0001
DFI, g/d	83.57	83.92	84.14	83.67	83.25	0.53	0.267	0.258
FCR	1.668	1.572	1.592	1.593	1.579	0.01	<0.0001	<0.0001
Mortality (%)	7.50	6.81	4.61	6.73	7.71	1.13	0.116	0.115

[1]Dietary total starch provided by the different cultivars; Marius1-P25, Marius1-P50 = diet containing peas at inclusion percentage that supplied 25% and 50% of total dietary starch, respectively. [2]DG = daily gain; DFI = daily feed intake; FCR = feed conversion ratio. [3]Each mean represents 8 experimental units of 6 broiler chickens each.. [4]KDS of Marius1-P25 and Marius1-P50 calculated with a KDS for peas of 1.03 h-1 (Weurding et al., 2001).

The digestion pattern of dietary CP was not affected by either wheat cultivar or origin, which is in concordance with results previously found (Gutierrez del Alamo et al., 2008b). This could be due to both the low contribution of the different wheat samples to dietary CP (average 30%) and the great amount of the endogenous protein in the ileal flow (4.4 g/kg DM intake, Golian et al., 2008).

Broiler performance was affected by KDS in a quadratic way. The greatest animal growth was observed at KDS around 2.2 h^{-1}. This level was reached by chickens fed the ST coming from Isengrain (100% of dietary ST) or by a mixture of ST coming from Marius1 (75% of dietary ST) and peas (25% of dietary ST). The worst broiler performance was observed with KDS 1.8 h^{-1} (50% dietary ST from Marius1 and 50% from peas). Therefore, KDS has a direct relation with animal performance similar to results obtained by Weurding (2002). In his study, better FCR was observed with KDS values closer to 1.26 h^{-1} whereas in ours a KDS value of 1.80 h^{-1} impaired performance.

Figure 2. Blood glucose response1 (mg/dl) from broilers fed diets containing a rate of starch digestion (h^{-1}) of 1.80 (•), 2.17 (▫), 2.20 (o), 2.48 (◆) and 2.56 (▵)..

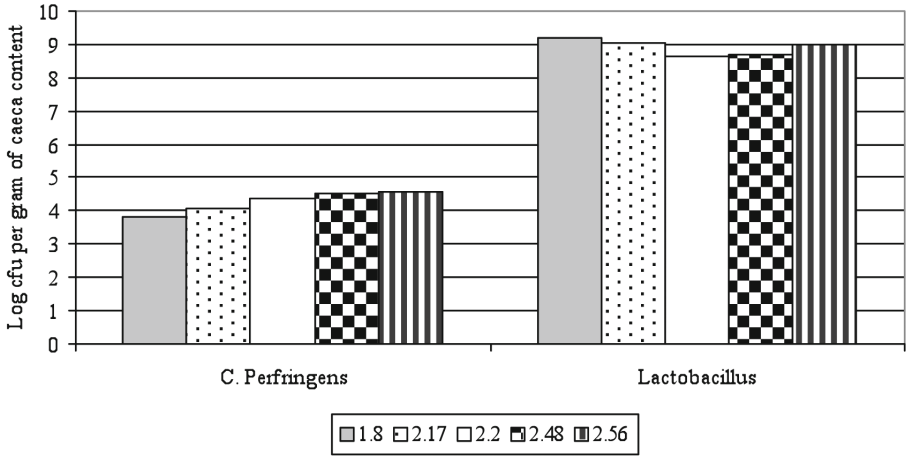


[1] *Each mean represents 10 replicates.*

According to Van den Borne et al. (2007) pig performance is maximized when the absorption of energy providing nutrients and amino acids for protein synthesis is synchronized. Black et al. (2005) proposed an asynchrony in the timing of absorption of amino acids and glucose as an explanation to the lower energy utilization from sorghum than from wheat based diets in

poultry. In our experiment, although ST and dietary CP were digested at similar rates (2.29 and 2.21 h^{-1}, respectively), the time needed to digest 50% of both was different. Thus, 50% of the ST was digested as average at 32.43 min after arriving the duodenum whereas 50% of the CP was digested at 52.98 min as average. This may indicate that reducing the KDS of a diet will supply gradually ST along the small intestine where it can be better synchronized with dietary CP supply. The lower utilization efficiency of the diet with KDS 1.8 h^{-1} could be due to the influence of other factors like CP digestibility or the different rate of amino acid absorption. In our work, experimental diets were formulated to contain the same amount of digestible amino acids. However, this led to a low or none inclusion of synthetic lysine in diet with 50% peas which could affect the synchronization between glucose and the most limiting amino acids in broiler diets (Baker and Han, 1994).

Figure 3. Effect of diets containing different levels of rate of starch digestion (h^{-1}) on bacterial counts in the caeca of broiler chickens[1]



[1] *Each mean represents 8 replicates.*

The differences in performance observed in the present experiment were more pronounced during the starter than during the grower period. Daily gain increased (average 10% and 7.5%, starter and grower period, respectively) when KDS increased from 1.8 to 2.2 h^{-1} and decreased 5% (starter period) or was maintained (grower period) when KDS increased from 2.2 to 2.5 h^{-1}. This may be explained, in part, by the low digestive capacity (Nitsan et al., 1991) and therefore relatively low feed intake during the starter period as compared with the grower period. So, low KDS values in very young birds can be limiting. During the grower

period the animals increased DFI in an attempt to compensate for the negative effects of both low and great KDS.

Blood glucose release was measured for 420 min because total MRT from crop to cloaca had been reported to vary from 266 min (caeca not included, Van der Klis et al., 1990) to 433 min (caeca included, Shires et al., 1987). At 30 min after feeding the animals, blood glucose increased to 239 mg/dl (Figure 2), which may be explained by a fast transit time from crop to the small intestine due to wet tube-feeding after fasting (12 h). The maximum blood glucose response was obtained from 50 to 240 min after feeding which agrees with the MRT in the small intestine obtained in the current work (159.80 min). When glycemic index (from 60 to 65%) was calculated no differences among diets were found. This suggests that the glycemic index in broiler chickens does not reflect the differences in KDS contrary to what observed by Englyst et al. (1996) in humans. On the other hand these results confirm the isoenergetic level of diets.

No differences among diets in Clostridium perfringens and Lactobacillus were found in this experiment. This is likely due to the narrow KDS range among diets and the low number of replicates used.

In conclusion, the rate of ST digestion varies among wheats, depending on both genetic and environmental conditions of the grain, and affects broiler performance. Blood glucose release or glycemic index cannot be used to predict the KDS of a feed.

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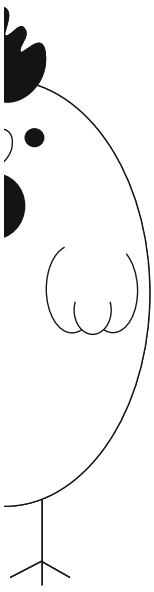
CHAPTER 7

General Discussion



INTRODUCTION

Current modern feed evaluation systems for poultry use nutrient compositions and their digestibility coefficients to estimate the energy value of a raw material (CVB, 2007). Nutrient compositions are determined by proximate analysis whereas digestibility coefficients are calculated by difference between intake and fecal output. In order to use feedstuffs for making feed, the nutrient compositions and digestibility coefficients of a feedstuff must be known. The data about this are usually taken from tables and used to compose the feed. Tabulated poultry feeding tables give values for nitrogen-corrected apparent metabolizable energy **(AMEn)**, starch **(ST)** content and ST digestibility coefficient (NRC, 1994; INRA, 2002; FEDNA, 2003; CVB, 2007). Therefore, formulations on a daily basis are done considering wheat a uniform raw material with differentiation among wheat batches not usually taken into account. However, this is too simplistic and inaccurate to be used in European broiler chicken diets since wheat is the main cereal used (up to 55-60%) and supplies most of the dietary AMEn (up to 65-70%). If the energy value of wheat is not accurately determined broiler may be fed with an unbalanced diet and, as a consequence, its performance can be negatively affected. Some research papers have shown that the nutritive value of wheat and its final utilization by the broiler depend on several factors which can be classified as intrinsic (cultivar, nutrient content and composition, etc) and extrinsic factors (growing conditions, post-harvest storage, etc) to the wheat grain (Carré, 1990; Classen et al., 1995; Scott, 2000, 2002; Jones and Taylor, 2001; Preston et al., 2001; Steendfelt, 2001; Pirgozliev et al., 2003). Obviously, an accurate knowledge of how much energy is supplied by wheat and the factors that influence it, is important in terms of optimizing feed formulations and maximizing broiler performance.

The objectives of the present thesis were to determine the variability of wheat samples received at the feed mills in both physico-chemical properties and AMEn content. Afterward, the factors that influence wheat AMEn and animal performance were studied with special focus on ST as the main energy component of wheat. Chapters 3, 4 and 5 were destined to investigate variations in wheat nutritive value. Additionally, in Chapter 4 the relationship between wheat nutritive value and animal performance was evaluated. In Chapter 6 the relationship between ST digestion rate of wheat and animal performance was investigated (Table 1).

Table 1. Chapters where the effect of the studied factors on the different variables was investigated

Factors[1]	Ileal starch digest.	Fecal starch digest.	Starch digestion rate	Dietary AMEn	Dietary ileal CP digest.	Growth performance
Wheat cultivar	4,5,6	4,5,6	5,6	4,5	4,5,6	4
Wheat cultivar origin	6	6	6	-	6	6
NSP-degrading enzymes	4	4	-	4	4	4
N fertilization	5	5	5	5	5	-
Wheat chemical composition	3	3	-	3[2]	3	-
Starch digestion rate	-	-	-	-	-	6

[1] AMEn = nitrogen-corrected apparent metabolizable energy; CP = crude protein; NSP = non-starch polysaccharides; N = nitrogen. [2] Wheat AMEn.

VARIABILITY AMONG WHEAT SAMPLES

Variation in Physico-Chemical Properties

Several research papers through years have demonstrated that wheat is a variable raw material (Chapter 2). The amount of nutrients in wheat grain and its physical properties depend on several factors such as wheat variety, environmental conditions during crop growth and post-harvest storage (Batthy et al., 1974; Jood et al., 1993; Wiseman, 2000; Rose et al., 2001; George and McCracken, 2003; Kim et al., 2003). For example, ST content may vary up to 15% (on a DM basis) between batches (Kim et al., 2003) and crude protein **(CP)** can range from 8.9 to 18.3% (Choct et al., 1999). Physically, specific weight **(SW)** can have values between 69.5 and 80.0 kg/hl (Wiseman, 2000) and viscosity can range between 1.8 to 5.3 cps (Classen et al., 1995).

All the above mentioned research has been usually performed with very limited number of wheats where samples are specifically selected to differ in one or two parameters. However, wheats received at the feed mills are normally a mixture of different wheat cultivars, collected from different areas and stored for an unknown number of weeks or months. Moreover, wheats with bakery properties are sometimes delivered to the feed industry due to an excess in their production or when some bakery quality parameter is not correct. Therefore our first objective was to find out which is the real variation in the wheats used in European feed mills

(Chapter 3). For that purpose, we collected 50 wheat samples from 5 different countries in Europe. Samples were collected after they were considered good for feed production (feed mill quality control passed). The collected samples differed mostly in viscosity and CP content. Surprisingly, we found that within wheat batches there was constant ST content (CV = 3.8%) which can be proper mixing and a good quality control within the feed mill. In the feed industry, SW is considered one of the parameters defining wheat quality with an agreed arbitrary minimum of 72 kg/hl for feeding quality wheats (McCracken et al., 2002). Specific weight is normally measured in the feed mills because it is a simple procedure and there is a clear relation between SW and ST content (Hickling, 1994; McCracken and Quintin, 2000; McCracken et al., 2002). Therefore, wheats received at the feed mill should not be expected to be very variable in ST content.

Variability in Nitrogen-Corrected Apparent Metabolizable Energy

The AMEn of different wheat batches vary, with reported values ranging from 2,628 to 3,800 kcal/kg dry matter **(DM)** (Mollah et al., 1983). Wiseman (2000) suggested that the variation observed for wheat AMEn can be due to a multitude of reasons connected to factors within the wheat grain. Therefore, we used many wheat samples (50 samples) to test the variability in AMEn of wheats inside Europe. We found more than 700 kcal/kg DM difference in AMEn among different batches of wheat which agrees with results obtained by other authors (Veldman and Vahl, 1994; Classen et al., 1995). Most of the samples (56%) showed an AMEn value between 3,200 and 3,400 kcal/kg DM but 18% of them showed a very low energy value and felt into the low-AME category of wheat (< 3,100 kcal/kg DM). This confirmed that the low-AME phenomena is not only confine to Australian or Canadian wheats (Classen et al., 1995; Veldman and Vahl, 1994; Wiseman et al., 2000; Svihus and Gullord, 2002). Additionally, the mean determined wheat AMEn value in this project was lower than the one reported in current feeding tables (Table 2). This means that current feeds for broilers are formulated with an overestimation of the energy provided by the wheat. The overestimation is even higher in young birds than in older ones as dietary energy digestibility is lower in young animals (Salih et al., 1991; Santos et al., 2004; Chapter 3). All together clarifies and quantifies the big risk that the nutritionist is taken when using tabulated feeding values for wheats in general and for low-AME wheats in particular.

Scientists have focused their research on finding the factors that define wheat AMEn variability and on finding technological tools that may reduce such a variation. One of the worldwide recognized factors influencing wheat AMEn variability is the non-starch polysaccharides **(NSP)** of wheat, (mainly soluble arabinoxylans) which cannot be degraded by the endogenous host enzymes (Choct and Annison 1990, 1992a).

Table 2. Chemical composition (% DM[1]) and AMEn[1] (kcal/kg DM) of wheat as it appears in feeding tables or determined in the present thesis

Starch[2]	Crude Protein	Crude Fiber	Ether Extract	AMEn	Reference
-	12.92	3.37	2.80	3,506	NRC, 1994
69.70	12.10	2.53	1.73	3,433	INRA, 2002
66.59	12.64	3.16	2.03	3,555	FEDNA, 2003
67.62	12.78	2.76	1.49	3,304	CVB, 2007
66.16	13.31	2.65	1.68	3,262	Current thesis

[1] *DM = dry matter; AMEn = nitrogen-corrected apparent metabolizable energy.*

[2] *Ewers.*

Feed industry reacted to these findings on wheat NSP by adding NSP-degrading enzymes and expected that as a result there would be an increase in nutrient digestibility and in AMEn and that also AMEn variability would be reduced (Svihus and Gullord, 2002; Choct el at., 2006). This assumption could not be corroborated by us (Chapter 4) since we found that the effect of added enzymes did not increase nutrient digestibility and dietary AMEn in all wheat samples (Table 3). Similarly, in other experiments we found that variability in AMEn among wheat samples was affected by cultivar and also by crop nitrogen fertilization (Table 3).

Prediction of AMEn Content of Feed Wheat Samples by Their Physico-Chemical Properties and Starch Digestibility

There are several equations proposed to predict the AMEn of a specific ingredient but its precision is less than the prediction of AMEn in the whole diet. Factors as the low variation among the nutrient composition between batches in the same raw material and variations in their digestion difficult the efficacy of the prediction (Carré and Rozo, 1990). Additionally, ingredient AMEn equations are normally based on physico-chemical properties of the ingredient and not on their digestibility properties itself. This avoids making expensive digestibility trials with animals. Moreover, wheats received at the feed mill are usually totally used within two to three days. Therefore, the prediction of wheat AMEn by an equation that can be directly applied by the nutritionist in formulation of the diet should include terms that can be easily and rapidly measured upon arrival of the sample. In general, the terms in the equation are chemical parameters measured via near-infrared spectroscopy.

Huyghebaert and Schöner (1999) found a coefficient of determination of 0.49 when AMEn of wheats was predicted only by ST. The same authors found a coefficient of determination up to 0.63 when both ST and measured viscosity (Carré et al., 1995) were introduced into the equation. We failed to obtain a valid equation when introducing only ST into the equation (R^2 = 0.17, P = 0.0025; RSD = 142 kcal/kg DM). When we used stepwise regression viscosity trait was not even chosen.

Starch digestibility greatly influences wheat AMEn (Mollah et al., 1983; Rogel et al., 1987; Wiseman et al., 2000). As discussed by Longstaff and McNab (1986) the term digestible ST is what best define wheat AMEn. If digestible ST was introduced into the equation together with the physico-chemical properties of wheat the best determination coefficient obtained was 0.58 (RSD = 118 kcal/kg DM). The digestibility coefficients of other nutrients in the wheat were not assessed in the present thesis. Taken into account that wheat can provide as much as 70% of the dietary metabolizable energy requirements for broilers (McNab, 1996) a coefficient of determination of 0.58 seems to be not accurate enough to be used by feed mills and nutritionists. With these results in mind we started to wonder which were the factors that most influence wheat AMEn and how could we measured and quantified them.

FACTORS AFFECTING THE NUTRITIVE VALUE OF WHEAT

Wheat Cultivar

Certain physical and chemical characteristics of wheat such as viscosity, hardness, starch size and shape or total NSP change according to cultivar (McNab and Knox, 1999; Carré et al., 2002; Kim et al., 2002; Morris, 2002). Moreover, it has been shown that some of those genetically determined characteristics affect wheat AMEn for broiler chickens (Carré et al., 2005). With this in mind we wanted to know if the classification of wheat samples based on cultivar could improve the prediction of the nutritive value and utilization of wheat grains by broiler chickens. This question was answered in three different experiments. In the first experiment we used different wheat cultivars grown in the same area but with unknown growing conditions (i.e. fertilization, treatments) (Chapter 4). In the second experiment wheat cultivars were grown in the same area but at two different nitrogen fertilization levels (Chapter 5). Finally, in the third experiment, 3 wheat cultivars were grown in the same area with similar procedures whereas one of them was grown in a different location (Chapter 6).

Table 3. Effect of the different variables investigated on the nutritive value and utilization of wheat by broiler chickens

Factors Studied[1]	Ileal starch digestibility (%)	Fecal starch digestibility (%)	Starch digestion rate (h^{-1})	Dietary AMEn (Mcal/kg DM)	Dietary Ileal CP digestibility (%)	Growth performance (g/d)
Wheat cultivar						
Chapter 4	97.9	98.5	-	2.93 vs. 3.07	80.0	45.9 vs. 47.7
	ns	ns	-	P < 0.001	ns	P < 0.001
Chapter 5	97.0	98.0	2.45 vs. 3.28	3.24 vs. 3.33	79.7 vs. 81.8	-
	ns	ns	P < 0.001	P < 0.001	P < 0.05	
Chapter 6	95.6	96.3 vs. 98.1	2.17 vs. 2.56	-	79.7	52.5 vs. 53.4
	ns	P < 0.05	P < 0.05	-	ns	P < 0.001
Wheat origin	95.6	96.7	1.96 vs. 2.56	-	80.7	-
(Chapter 6)	ns	ns	P < 0.001	-	ns	-
NSP-degrading enzymes (Chapter 4)						
Main effect	97.9	98.5	-	3.00	80.0	46.9
	ns	ns	-	ns	ns	ns
Interaction cultiv. x enz.	ns	ns	-	2.9 vs. 3.1 P = 0.001	ns	ns
N fertilization	97.0	98.0	2.79	3.22 vs. 3.32	80.9	-
(Chapter 5)	ns	ns	ns	P < 0.001	ns	-
Starch digestion	-	-	-	-	-	*50.1 vs. 53.4*
rate (Chapter 6)	-	-	-	-	-	P < 0.001
Wheat chemical	CP (r = -0.61)	CP (r = -0.57)	-	ST[2] (r=0.42)	-	-
composition (Chapter 3)	ST (r = 0.44)	ST (r = 0.47)	-	CP[2] (r = -0.28	-	-

[1] AMEn = nitrogen-corrected metabolizable energy; CP = crude protein; NSP = non-starch polysaccharides; N = nitrogen. [2] Wheat AMEn

Our results revealed that wheat AMEn and broiler performance were greatly influenced by wheat cultivar. Maximum dietary AMEn differences among wheat cultivars were 140 kcal/kg DM (Table 3) which, if transformed into wheat AMEn differences, they would represent about 220 kcal/kg DM. Unfortunately, in terms of AMEn when added enzymes or nitrogen fertilization were used wheat cultivars did not respond in a uniform fashion. This result agrees with Wiseman (2000). One intriguingly result was that wheat cultivar did not affect ileal and fecal ST digestibility but it did affect ST digestibility in the different parts of the small intestine (proximal jejunum **PJ**; distal jejunum, **DJ**; proximal ileum, **PI** and distal ileum, **DI**), mainly in the upper part of the small intestine. Moreover, the rate of ST digestion was affected by wheat cultivar with some cultivars being digested faster than others (Chapter 5). However, this effect was not corroborated in Chapter 6 where the effect of origin (1.96 vs. 2.56 h^{-1}) was greater than that of wheat cultivar (2.17 vs. 2.56 h^{-1}). The information refering the same wheat cultivar grown at two different locations with regard to rate of ST digestion is very limited and needs further investigation. As a whole, our results suggest that the nutritive value and utilization of wheat by broilers in relation to wheat cultivar is in increasing order: nitrogen crop fertilization--wheat cultivar--growing location.

Non-Starch Polysaccharides Degrading Enzymes

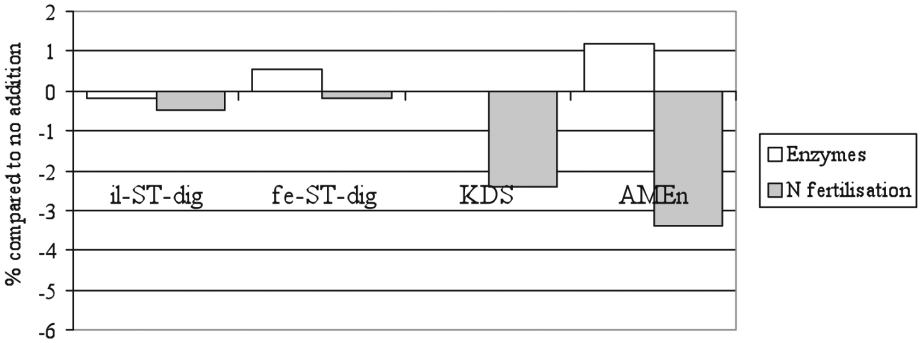
The fiber fraction of the wheat grain is composed by an outer layer of bran and an inner layer of endosperm. The NSP content in the wheat bran consists of 64 to 69% arabinoxylans and in addition 15 to 31% cellulose (Ring and Selvendran, 1980; Southgate and Englyst, 1985). The NSP in wheat endosperm are nearly all (88%) arabinoxylans (Mares and Stone, 1973a). The arabinoxylans of the bran are insoluble in water because they are bound to the cell walls by alkali-labile ester-like cross links (Mares and Stone, 1973b). In contrast to this, part of the arabinoxylans in the aleurone and the endosperm of wheat, which are not bound to the cell walls, are soluble in water (Mares and Stone, 1973b). The soluble arabinoxylans can absorb as much as ten times their weight in water and form viscous solutions (Choct, 1997).

Even when present at very low levels the NSP of cereals possess antinutritive activity as shown by Choct and Annison (1990, 1992a,b). In wheat, the negative effect of the NSP on nutritive value seems to come from the soluble fraction of the arabinoxylans (Van der Klis and Van Voorst, 1993; Almirall and Esteve-García, 1994; Angkanaporn et al., 1994; Schutte et al., 1995; Yasar and Forbes, 2000). The viscous nature of soluble arabinoxylans has a direct impact on nutrient utilization because they slows down the rate of passage of food through the intestinal track. As a result, the interaction with the gut

microflora is increased and this can affect the physiology and morphology of the digestive tract (Van der Klis and Van Voorst, 1993; Almirall and Esteve-García, 1994; Yasar and Forbes, 2000). Consequently, nutrients as ST, CP or lipids are not well digested and less of their nutrients can be absorbed (Annison, 1991; Van der Klis et al., 1993). On the other hand, the insoluble NSP fraction seems to have very little or no effect on nutrient utilization and on AMEn of the wheat in monogastric animals (Carré, 1990; Annison, 1991; Angkanaporn et al., 1994). However, our data showed that both the soluble and the insoluble NSP have a negative impact on dietary AMEn in agreement with Steenfeldt (2001). Moreover, when the viscous properties of soluble arabinoxylans were reduced by the addition of enzymes, dietary AMEn negatively related to the insoluble NSP.

The NSP of plant origin are not digested by host (broiler) endogenous enzymes. To overcome this problem, wheat based diets for poultry nearly always include exogenous NSP-degrading enzymes with the purpose of increasing nutrient digestibility and reducing the variation in nutrient digestibility (Bedford, 2000). This in turns means improvements in performance, nutrient utilization and AMEn of broilers (Schutte et al., 1995; Scott et al., 1998; Mathlouthi et al., 2002; Engber et al., 2004; Scott, 2005). In poultry nutrition there is a common conception that the use of NSP-degrading enzymes will reduce the variability among wheat grains in AMEn content and will equalize the nutritive value of different wheat samples by eliminating the viscous properties of the NSP. Moreover, sometimes in practice the AMEn content of wheat is increased by 5% into diet formulation when NSP-degrading enzymes are included. However, we wondered if this is a correct and accurate estimation of the beneficial effect of the addition of NSP-degrading enzymes into wheat-based diets. To answer this, we run an experiment in which four wheat cultivars were supplemented with a viscosity-reducing enzyme or not and fed them to broiler chickens during the whole production cycle (Chapter 4). Our data showed that exogenous enzymes do not increase the nutritive value of wheat grains in general (Figure 1) although they are able to increse the nutritive value of some wheats (Chapter 4). On the contrary the effect of the viscous-reducing enzyme is wheat cultivar dependent and seems to be related to the amount of soluble NSP present in the wheat grain and their capacity to increase viscosity. Therefore, the 5% extra AMEn by the use of enzymes requires precaution. Choct and Annison (1992b) suggested that birds can tolerate up to 35 g of total pentosan/kg DM and Steenfeldt (2001) suggested that the arabinoxylans content of wheat becomes detrimental to the chickens when the concentration reaches certain level. To calculate that level further research is needed.

Figure 1. Effect of the addition of NSP degrading enzymes (white bar) or N fertilisation (grey bar) on ileal ST digestibility (il-ST-dig), fecal ST digestibility (fe-ST-dig), rate of ST digestion (KDS) and AMEn in broiler chickens of 27 d.



Nitrogen Fertilization (CP content)

Crude protein content of the wheat negatively correlates with ST content (Svihus and Gullord, 2002; Chapter 3). This indicates that a high protein content was associated with small and incompletely filled endosperm cells (Svihus and Gullord, 2002). Since ST content is the main energy supplier of the wheat (Svihus and Gullord, 2002; Carré et al., 2005; Chapter 3) it may be expected that wheats with high CP content show lower AMEn. This was shown by Svihus and Gullord (2002) and it was also found by us in Chapter 3. However, in the same study we observed that CP content clearly affected ST digestibility at both the ileal and fecal level. Therefore, the negative effect of CP on wheat AMEn is not fully explained by a reduction in wheat ST content but also by a reduction in ST digestibility.

The starch granules in the endosperm are embedded in a protein matrix (Hoseney, 1998). In wheat, the interaction between protein and starch is what defines hardness (Barlow et al., 1973). According to Morris (2002) the fiabrilin protein represents the molecular basis of wheat grain hardness. However, other proteins are also involved in hardness (Amiour et al., 2002). Wheat hardness has been shown to depend strongly on cultivar and, in part, on protein content (Oury et al., 1998). Several papers have shown wheat hardness as a factor which influences wheat AMEn and broiler performance (Rose at al., 2001; Pirgozliev et al., 2003; Carré et al., 2005). An hypothesis behind this is an inhibitory effect of the protein matrix on digestibility and availability of ST in the endosperm of wheat particles. Two experiments were run to study this (Chapters 5 and 6). In both Chapters we studied the ST digestibility in four different segments of the small intestine (PJ, DJ, PI and DI) and in overall (at fecal level). Afterwards, the rate of ST digestion was calculated. In Chapter 5, the differences in CP content of the wheat samples were caused by different crop nitrogen fertilization (Uhlen

at al., 2004). However, in Chapter 6 those differences were associated mainly with cultivar and with location of growth. Nitrogen fertilization did not affect ST digestibility in any of the segments studied (Chapter 5) but it reduced dietary AMEn by 2.4% (Figure 1). Moreover, nitrogen fertilization increased the range in dietary AMEn confirming its negative impact in the energy value of the wheat samples.

In our study on how nitrogen fertilization (CP content) influences the rate of ST digestion we found no relation in Chapter 5. However, contrary to this finding we found a relation between CP content and rate of ST digestion when the CP content was from different cultivars (Chapter 6). When data from both experiments were combined the correlation dropped dramatically. This seems to indicate a different effect of genotype and of environment. In fact, the genetically defined CP content of wheat seems to be what mostly define the effect of CP on ST digestion.

Starch

Wheats are introduced in poultry diets as an energy source. Starch is by far the largest component of the mature wheat grain (up to 73% of its DM content, Pomeranz and MacMasters, 1968) and the largest contributor to the energy content of the wheat. Therefore, any variability in its content and digestibility will affect wheat AMEn and consequently, it will affect animal performance.

Starch Content and Starch Digestibility. A positive relationship between ST content and wheat AMEn has been repeatedly shown (Huyghebaert and Schöner, 1999; McCracken and Quintin, 2000; Svihus and Gullord, 2002; Chapter 3). This positive relationship should be expected since ST is the major energy component of wheat and its digestibility is usually very high (range between 93% and 98%) (Annison, 1990; Choct et al., 1999; Carré, 2004; this thesis). However, some research papers have reported wheat ST digestibilities below 82% (Rogel et al., 1987; Mollah et al., 1983; Wiseman et al., 2000; Svihus 2001). In those cases, varation in ST digestibility rather than in ST content was the parameter that related most with variation in wheat AMEn. Rogel et al. (1987) found that isolated wheat ST was entirely digested by broiler chicken whereas it was not (< 82%) when fed in situ. Svihus (2001) concluded that the major cause for low-ME wheats was a low ST digestibility. Accordingly, it seems that is not ST per se what is poorly utilized in broiler chickens but that other factors are responsible for reducing the ST digestibility. In all our experiments we measured ST content and ST digestibility at both ileal and fecal level and found that ST digestibility was always rather high (average 95%) which confirmed that broilers digest well wheat ST (Annison, 1990). The variable most related to ileal and fecal ST digestibility was CP (negative

relationship). In Chapter 3 we found that ST content was the nutrient most related to wheat AMEn but we could not relate dietary AMEn to broiler performance. This finding agrees with others (Steendfelt, 2001; Scott, 2000, 2002).
No major differences between ileal and fecal ST digestibility were found in this thesis and also not in other research papers (Yuste et al., 1991; Steenfeldt et al., 1998; Weurding, 2002). This means that there is no ST fermentation in the caeca. Two possible explanations for this could be; i) the absence of amylolytic bacteria in the caeca or ii) the undigested ST granules do not enter into the caeca. In another experiment (Chapter 6) we found a no significant reduction of the total number of Clostridium Perfringens when animals were fed with a diet with low ST digestion rate. This effect could be explained by a greater load of ST into the ileum which may have provoked a shift from protein to ST degrading bacteria.
According to Riesenfeld et al. (1980) the major part of ST content in a diet is digested in the duodenum and the digestion of ST is normally completed in the jejunum. Waldron (1997) observed that most of the differences in ST digestion among wheats occur in the upper part of the small intestine. We observed around 82% of the ST digested by the time digesta reacheed the ileum and 95% of it already digested in the PI (Chapters 5 and 6). Differences among wheat samples were found in the upper part of the small intestine (jejunum) but not in the ileum and excreta.

Starch Digestion Rate. The ST digestion rate in the small intestine of broiler chickens differs among feedstuffs (Weurding, 2002) and among wheat samples (Chapters 5 and 6). Rate of ST digestion determines rate of glucose absorption but it cannot be predicted by blood glucose measured in broiler chickens (Chapter 6). Moreover, rate of ST digestion cannot be defined by site of ST digestion because apart from place of ST digestion, digesta transit time and digesta capacity affects rate of ST digestion. If diets stay longer in the upper part of the small intestine the digestion of ST will be greater because enzymes will have more time to act. On the other hand, if digesta transit time is greatly reduced, intake decreases and as a consequence animal performance will be impaired. Weurding (2002) found that chicken performance was affected by ST digestion rate within a certain range ($1.26 <$ rate of ST digestion < 3.27 h^{-1}). Inside that range slowly digestible ST improved performance. When rate of ST digestion is modified by the use of different wheat cultivars broiler performance is also modified regardless of the level of ileal or total ST digestibility of the wheat cultivars (Chapter 6). The response seems to be both linear and curvilinear. In addition, the effect seems to indicate a narrow range in rate of ST digestion in which performance is maximized. The effect is even more pronounced in young than in older broiler

chickens and can be explained by the limited digestion capacity of young birds (Nitsan et al., 1991). This indicates that broiler performance is very sensitive to rate of ST digestion and that wheat ST digestion rate is an important variable in broiler performance.
In addition we measured dietary CP digestion rate and found that it was digested slower than the ST. Therefore, part of the explanation of the effect of rate of ST digestion in broiler performance could be a better synchronization with the dietary CP rate of digestion which will lead to a more efficient growth.

IMPLICATIONS

Wheat is (and most probably will be in the future) the most used cereal in broiler nutrition in Europe. Poultry industry normally receives wheat grains at the feed factory in trucks. Usually, the trucks transport a mixture of different wheat cultivars collected from different areas and mixed during storage. It has been clear from our studies in this thesis that wheat cultivar can greatly affect animal performance and AMEn. Therefore, the difference in cultivar with regard to wheat utilization has to be taken into account in diet formulation. It is therefore advised to transport wheats to the feed factories separate by cultivar. Although there is still part of the variation between wheat cultivars which is not identified and which cannot be applied, a separate transportation to the factory will reduce variability in AMEn and animal performance.
We found that the use NSP-degrading enzymes do not always have a clear effect on the energy content of the wheat. Therefore, the increase of the energy supplied by the wheat when enzymes are added into the diet is not always recommended. In this way, the use of NSP-degrading enzymes can be considered as a tool to ensure a non increase of the viscosity of the digesta in the gut of the broiler which may lead to poor nutrient utilization.
The rate by which wheat ST is digested by broiler chickens differs among wheat cultivars and influence animal performance. Its consideration in dietary formulation is more important than the consideration of total ST digestibility.

FUTURE RESEARCH

From this thesis it has been shown that NSP-degrading enzymes are not always increasing the nutritive value and utilization of the wheat grains by the broiler chickens. It would be interesting to know which are the factors related to the NSP fraction of the wheat grain that determine the effectiveness of the added enzymes. With that knowledge the use of NSP-degrading enzymes in the diet can be optimized.

Wheat cultivar affects the nutritive value and the utilization of wheat by broiler chickens. However, some physico-chemical properties of the wheat cultivar are affected by external factors such as growing origin. It may be worthwhile to determine factors that being genetically determined are not affected by external agents.
We observed that ST digestibility differences were more pronounced in the upper than in the lower part of the small intestine. Moreover, rate of ST digestion differed among wheat grains and affected animal performance. From a practical point of view it would be interesting to find new in vitro tools that could easily measure rate of ST digestion. Among all, an in vitro method or the use of the use of near-infrared spectroscopy would be of great use.
Finally, synchronization among energy and protein seems to be the key point for maximizing broiler performance. It is highly advisable to further investigate which should be the level of synchronization and how to achieve that in the diet. With this, broiler nutritionist would talk about energy to protein synchronization rather than about a balanced ratio energy to protein.

CONCLUSIONS

Main conclusions from these studies were as follows:

- Wheat energy value is not accurately predicted from its physico-chemical properties. Among all the properties studied, starch content was the variable most related to the energy value of wheat.

- Starch digestibility of wheat by broiler chickens is negatively related to wheat crude protein content.

- The nutritive value and utilization of wheat is affected by cultivar. The effect of exogenous enzymes used in this study on dietary nitrogen-corrected apparent metabolizable energy was cultivar dependent and related to digesta viscosity.

- Starch from wheat is mainly digested before the ileum with no further digestion after it.

- Starch digestion rate varies with wheat cultivar and some environmental factors (i.e. origin). Nitrogen crop fertilization does not influence starch digestion rate of wheat grains.

- Starch digestion rate affects animal performance, mainly in young animals.

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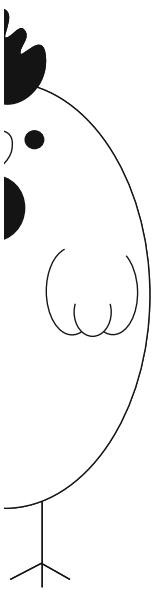
SUMMARY



Wheat is the most common raw material used in broiler nutrition in Europe, Australia and Canada. Although considered a moderately uniform ingredient, evidence has shown that the nutritive value of wheat for broilers varies considerably and it is affected by several intrinsic (cultivar, nutrient content and composition, etc) and extrinsic factors (growing conditions, post-harvest storage, etc). This variation is of importance to the feed industry whose primary objective is to optimize the efficiency of poultry production. An accurate knowledge of the nutritional value of wheat is essential to compose well balanced diets to animals.

Research over years has focused on the variation in nitrogen-corrected apparent metabolizable energy (**AMEn**) among wheat grains with special emphasis on the carbohydrate fractions (starch (**ST**) and non-starch polysaccharides (**NSP**)). The two main reasons for that are firstly, that wheat is essentially used as an energy source due to its high ST content; and secondly, that the NSP fraction of wheat negatively affects its nutritive value even when present at low quantities. The influence of ST on wheat AMEn depends on its content and digestibility whereas the influence of the NSP fraction on wheat AMEn depends mostly on the soluble NSP fraction and its capacity to increase the viscosity of the digesta in broiler chickens. The feed manufacturers have tried to counter-balance the variation in the nutritive value of wheat grains by measuring the ST content in receiving grains and also by adding NSP-degrading enzymes to the feed. The data for the coefficient of digestibility of the ST are usually taken from published tables. However nutritional tables give only means and they do not take into account the variation among wheat grains. So the factors associated with variation in the nutritive value between wheat samples are still not well understood and further research is required.

The aim of the present studies was to investigate the factors that influence AMEn of wheat and its utilization by broiler chickens. For that we ran digestibility as well as performance trials using the same group of broiler chickens. The studies were conducted with different wheat samples and cultivars collected from different origins or grown under different conditions. The variation in ST digestibility was studied by measuring the ileal and fecal ST digestibility as well as ST digestion rate along the intestinal gut. Digestibilities and AMEn values were measured by the marker technique using chromic oxide. The diets used in all experiments contained wheat as the only starch source and soya as the main protein source. This was done to mimic a typical European broiler diet. All diets met the nutritional requirements for broiler chickens at each age.

Prior to the experiments a literature review was conducted (Chapter 2). The review was split up in the study of physical and chemical factors that influence wheat nutritive value. At the end a brief description of the effect of the age of the broiler and its capacity to digest wheat was described. It was concluded that the nutritive value of wheat cannot be predicted by the traits measured so far in wheat samples. Additionally, the relationship between wheat AMEn and animal performance as well as the effect of NSP-degrading enzymes on equalizing the nutritive value of different wheat

samples was questioned. Crude protein (**CP**) content of the wheat and the effect of ST digestion rate and its synchronization with CP digestion were suggested as the major factors which influence the nutritive value of wheat.

The variation among wheat samples used in Europe was studied in Chapter 3. A total of 50 wheat samples were collected from 5 different countries in Europe (France, Ireland, Portugal, Spain and The Netherlands) after they had passed the quality control of the feed mill. First, the physico-chemical properties of the samples were measured under similar laboratory conditions. Second, each wheat sample was introduced into a feed and offered to broiler chickens individually allocated in cages from 21 to 30 d. Wheat AMEn, total ST digestibility and CP retention were measured in the excreta collected from 27 to 30 d whereas ileal ST and CP digestibilities were measured at d 30. The results showed that wheat samples varied most in viscosity (CV = 30%) and in CP content (CV = 18%) whereas ST content was more homogeneous (CV = 3.8%) than expected. Ileal and fecal ST digestibility were high (average 95.80%) and similar in range and in variation (CV = 2.4%). The CV of wheat AMEn was 5%. The average wheat AMEn was 7% lower than the value reported in the feeding tables. The CP content of the wheat was shown to negatively affect both ST digestibility and wheat AMEn. Starch content was the only parameter that positively affected wheat AMEn.

In Chapter 4 the effect of using NSP-degrading enzymes on the nutritive value and utilization of wheat was studied. We hypothesized that although the NSP-degrading enzymes might increase the nutritive value of wheat they were not able to equalize the response of cultivars. Four wheat cultivars and two levels (0 or 1 kg/t feed) of a viscosity-reducing enzyme blend were used in two separate experiments (growth and metabolic) conducted at the same time. In experiment 1, the experimental diets were offered to the animals during the whole growing-finishing period and ileal viscosity was measured at 4 consecutive weeks (starting at d 13). In experiment 2, ST and CP digestibilities and dietary AMEn were measured during both the starter and the grower period. We observed that wheat cultivars strongly influenced animal performance and dietary AMEn. The effect was most pronounced in young broilers. Starch digestibility was very high at both ileal and fecal levels (average 98.53%). The inclusion of the viscosity-reducing enzyme increased dietary AMEn by 1.1% in the starter period. Their effect in the grower period was linked to the wheat cultivar used, while the exogenous enzyme increased the dietary AMEn value of those cultivars with the highest soluble arabinose. Despite the observed positive effect of the viscosity-reducing enzyme on dietary AMEn, no differences in animal performance were observed. It was concluded that the use of a viscosity-reducing enzyme in this study was not able to eliminate the differences in the nutritive value of wheat cultivars.

Variation in ST digestion rate and its influence on animal performance was described in Chapters 5 and 6. In Chapter 5 two questions were addressed; first, the variation in ST digestion rate among

wheat cultivars and second the influence of nitrogen fertilization on ST digestion rate. Wheat cultivars were grown in the same field at two different nitrogen fertilization levels. Differences among wheat cultivars in ST digestion rate (maximum 25% difference) were observed despite the fact that total ST digestibility among cultivars was similar. The differences were caused mainly by differences in ST digestion in the upper part of the small intestine (jejunum). Nitrogen fertilization did not influence ST digestion rate.

In Chapter 6, we hypothesized that wheat cultivars could be classified based on their ST digestion rate and that ST digestion rate would impact animal performance. This was tested in two separate experiments. In the first experiment, a total of three (3) wheat cultivars, with one of them from two (2) origins, were used to determine their ST digestion rate. In the second experiment a total of five (5) different diets were all formulated to the same ST level by using three wheat cultivars alone and one cultivar mixed with peas. We observed a maximum difference in ST digestion rate of 15% among cultivars and of 23% between the same cultivar grown at 2 different locations. Animal performance was affected by ST digestion rate in a linear and quadratic way. The best animal performance was obtained with ST digestion rate around 2.2 h^{-1}. This effect was more pronounced during the starter than during the grower period. Blood glucose response changed with time but there was no effect of dietary treatments on blood glucose levels; therefore it could not be used to predict animal performance based on ST digestion rate. *Clostridium perfringens* counts did not differ among treatments likely due to the low number of replicates used. However, a non significant reduction in *Clostridium perfringens* counts was observed with the lowest ST digestion rate.

Main conclusions from these studies were as follows:

- Wheat energy value is not accurately predicted from its physico-chemical properties. Among all the properties studied, starch content was the variable most related to the energy value of wheat.

- Starch digestibility of wheat by broiler chickens is negatively related to wheat crude protein content.

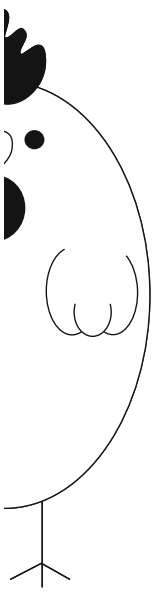
- The nutritive value and utilization of wheat is affected by its cultivars. The effect of exogenous enzymes used in this study on dietary nitrogen-corrected apparent metabolizable energy was cultivar dependent and related to digesta viscosity.

- Wheat starch is mainly digested before the ileum with no further digestion after it.

- Starch digestion rate varies among wheat cultivars and some environmental factors (i.e. origin). Crop nitrogen fertilization does not influence starch digestion rate of the wheat grains.

- Starch digestion rate affects animal performance, mainly in young animals.

SAMENVATTING



Tarwe is de meest gebruikte grondstof in de voeding van vleeskuikens in Europa, Australië en Canada. Het heeft een hoog gehalte aan zetmeel en is een belangrijke bron voor metaboliseerbare energie (nitrogen-corrected Apparent Metabolisable Energy, AMEn). De laatste jaren is echter duidelijk geworden dat de voederwaarde van tarwe aanzienlijk kan variëren. Dit wordt veroorzaakt door verschillende intrinsieke (ras, samenstelling, etc.) en extrinsieke factoren (groeiomstandigheden, opslagcondities, etc.). Kennis over de variatie in voederwaarde is van groot belang voor efficiënt gebruik van tarwe en het nauwkeurig kunnen samenstellen van pluimveevoeders.

Eerdere studies naar de oorzaken voor de variatie in voederwaarde hebben zich vooral gericht op de invloed van zetmeel en de niet zetmeel polysacchariden fractie (Non Starch Polysaccharides, NSP). De energiewaarde van tarwe wordt vooral bepaald door het gehalte aan zetmeel en de verteerbaarheid daarvan. Daarnaast kunnen specifieke, oplosbare NSP een anti-nutritioneel effect hebben doordat ze de viscositeit van de darmhinhoud verhogen en het verteringsproces en de darmflora bij kuikens verstoren. Nutritionisten proberen met beide aspecten rekening te houden door de chemische samenstelling van tarwe te bepalen, o.a. zetmeel, en door enzymen aan het voer toe te voegen die de NSP afbreken. Voor het berekenen van de voederwaarde wordt vervolgens gebruik gemaakt van standaardwaarden voor de verteerbaarheid (de 'tabelwaarden'). Dit zijn gemiddelde waarden die bepaald zijn in eerdere verteringsproeven. Een tekortkoming in deze benadering is dat geen rekening wordt gehouden met de variatie in verteerbaarheid tussen verschillende partijen tarwe.

Het doel van dit proefschrift is het vinden van verklarende en voorspellende factoren voor de AMEn van tarwe bij vleeskuikens. Hiervoor zijn verterings- en voederproeven uitgevoerd met kuikens met tarwemonsters van verschillende variëteiten, herkomst en groeiomstandigheden. De variatie in zetmeel verteerbaarheid is bepaald door het meten van de faecale en ileale zetmeelverteerbaarheid, alsmede door het bepalen van de snelheid van de zetmeelvertering. De verteerbaarheid van nutriënten en de AMEn is gemeten met chroomoxide als marker. De proefvoeders hadden tarwe als enige zetmeelbron en sojaschroot als de voornaamste eiwitbron. De samenstelling sloot aan bij wat in Europa gangbaar is en de voeders voldeden aan de nutriënten behoefte van vleeskuikens.

Het onderzoek is gestart met een literatuurstudie (Chapter 2) waarin de fysisch-chemische factoren zijn beschreven die van invloed zijn op de voederwaarde van tarwe. Aan het einde van dit hoofdstuk is het effect van leeftijd van het kuiken op de verteerbaarheid van tarwe beschreven. Geconcludeerd is dat de voederwaarde van tarwe niet nauwkeurig genoeg voorspeld kan worden met de tot dan toe gangbare methodes. Daarnaast lijkt de relatie tussen de AMEn van tarwe en groei en voederconversie van kuikens niet accuraat genoeg, evenals het effect van NSP afbrekende enzymen. Het eiwitgehalte van tarwe, het effect van de snelheid

van zetmeelafbraak en de synchronisatie van de zetmeel- met de eiwitafbraak leken de meest perspectiefvolle aanknopingspunten voor het verbeteren van de voorspelling van de voederwaarde van tarwe.

In een eerste grote studie (Hoofdstuk 3) is de variatie in voederwaarde onderzocht van in totaal 50 tarwemonsters uit 5 verschillende Europese landen (Frankrijk, Ierland, Portugal, Spanje en Nederland). Van deze monsters zijn diverse fysisch-chemische eigenschappen gemeten en is de energie-, zetmeel- en eiwitverteerbaarheid bepaald bij kuikens van 21 tot 30 dagen leeftijd. Van de fysische-chemische eigenschappen varieerde de viscositeit (CV=30%) en het eiwitgehalte (CV=18%) het meeste, terwijl het zetmeelgehalte meer homogeen was dan verwacht (CV=3.8%). De variatie in tarwe AMEn was 5%. De gemiddelde AMEn van tarwe was 7% lager dan de waarden die worden vermeld in voederwaardetabellen. Het eiwitgehalte van tarwe bleek een negatief effect te hebben op de zetmeelverteerbaarheid en de AMEn. Het zetmeelgehalte was de enigste parameter die een gunstig effect had op de AMEn.

In hoofdstuk 4 is het effect van NSP afbrekende enzymen op de voederwaarde van tarwe beschreven. De hypothese was dat NSP enzymen niet in staat zijn om de variatie in voederwaarde van tarwe op te heffen. Voor dit onderzoek zijn vier verschillende tarwevariëteiten getest, waarbij wel (1 kg per ton) of geen viscositeitverlagende enzymen aan het voer werden toegevoegd. Met de proefvoeders is een verterings- en een voederproef uitgevoerd. In de voederproef kregen de kuikens gedurende 4 opeenvolgende weken het proefvoer verstrekt (vanaf 13 dagen leeftijd) en de viscositeit van de darminhoud van het ileum (eind dunne darm) werd elke week gemeten. In de verteringsproef is de verteerbaarheid gemeten van energie, eiwit en zetmeel in twee perioden, de startfase en groeifase. Uit het onderzoek kwam naar voren dat de kwaliteit van tarwe een sterk effect heeft op de dierprestaties en de AMEn. De invloed was het grootst bij jonge kuikens. De zetmeelverteerbaarheid was hoog op zowel ileaal als faecaal niveau (gemiddeld 98.5%). Het enzympreparaat had in deze studie geen effect op de prestaties van de kuikens, ondanks het gegeven dat de viscositeit van de darminhoud werd verlaagd, de AMEn werd verhoogd met 1.1% in de start periode en een gunstig effect werd waargenomen op de AMEn van 1 van de 4 soorten tarwes in de groeiperiode. Alleen in de variëteit met de hoogste viscositeit verbeterde het enzympreparaat de AMEn.

De variatie in zetmeelverteerbaarheid en de invloed daarvan op dierprestaties is beschreven in Hoofdstukken 5 en 6. In hoofdstuk 5 is het effect van tarweras en van stikstofbemesting tijdens de teelt op de zetmeelverteerbaarheid tarwe bestudeerd. De twee tarwerassen werden geteeld in hetzelfde proefveld maar met twee niveaus van stikstofbemesting. Verschillen in verteringssnelheid van zetmeel werden waargenomen tussen de twee tarwerassen (maximaal 25% verschil) ondanks dat de zetmeelverteerbaarheid aan het eind van de dunne darm vergelijkbaar was. De verschillen in snelheid van zetmeelvertering waren m.n. aanwezig in het

voorste deel van de dunne darm (jejunum). Het niveau aan stikstofbemesting had geen invloed op de zetmeelvertering.
Vervolgens werd de hypothese geformuleerd dat de snelheid van de zetmeelvertering een belangrijke verklarende factor kan zijn voor de verschillen in de prestaties van de kuikens en een proef opgezet om deze hypothese te toetsen (Hoofdstuk 6). In het eerste experiment zijn in totaal 3 tarwe variëteiten, met één variëteit van twee herkomsten, onderzocht op verteringssnelheid van zetmeel. In het tweede experiment zijn in totaal 5 verschillende voeders samengesteld met 3 tarwe variëteiten. Eén tarwebron had twee herkomsten en werd daarnaast ook nog apart getest met erwten in het voer in plaats van sojaschroot. De voeders hadden een identiek zetmeelgehalte. Het verschil in de afbraaksnelheid van zetmeel was maximaal 15% tussen de variëteiten en 23% tussen de herkomsten. De afbraaksnelheid van zetmeel had een significante relatie met de prestaties van de kuikens. De beste groei en voederconversie werd gerealiseerd met een relatieve afbraaksnelheid van 2.2 per uur. Het effect was sterker in de startperiode dan in de groeiperiode. De bloedglucose respons veranderde met het toenemen van de leeftijd van de kuikens, maar er was geen effect van dieet. Ook het aantal Clostridium Perfringens in de dunne darm werd niet significant beïnvloed door het dieet, hoewel er numeriek minder Clostridium Perfringens werd waargenomen met de laagste afbraaksnelheid van zetmeel.

De belangrijkste conclusies in dit proefschrift zijn:

- De energiewaarde van tarwe bij kuikens wordt niet nauwkeurig voorspeld door de fysisch chemische eigenschappen. Van alle bestudeerde variabelen was zetmeel het meest gerelateerd aan het energiegehalte van tarwe.

- De zetmeelverteerbaarheid van tarwe heeft een negatieve relatie met het eiwitgehalte.

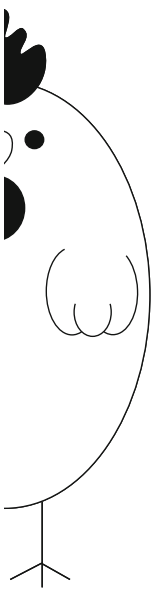
- De voederwaarde van tarwe wordt beïnvloed door de variëteit. Het effect van de in deze studie gebruikte enzymen op de voor stikstof gecorrigeerde energieverteerbaarheid was afhankelijk van variëteit en gerelateerd aan de viscositeit van de digesta.

- Het zetmeel van tarwe wordt voornamelijk verteerd vòòr het ileum.

- De afbraaksnelheid van zetmeel wordt beïnvloed door variëteit en herkomst van de tarwe. Het niveau aan stikstofbemesting had hierop geen invloed.

- De afbraaksnelheid van zetmeel heeft een effect op de prestaties van kuikens, m.n. in de startfase.

RESUMEN



El trigo es el ingrediente más habitual para piensos de pollos broiler en Europa, Australia y Canadá. Aunque se considera que es una materia prima poco variable, la evidencia, con el tiempo, ha demostrado que su valor nutritivo para los broilers varía considerablemente y depende de varios factores intrínsecos (variedad, contenido de nutrientes y composición, etc.) y extrínsecos (condiciones ambientales, almacenamiento, etc.). Esta variabilidad tiene importancia para la industria de los piensos compuestos, cuyo primer objetivo es optimizar la eficacia de la producción del pollo. Un conocimiento preciso del valor nutritivo del trigo resulta fundamental para poder fabricar piensos equilibrados para los animales.

Durante años, la investigación se ha centrado en la variabilidad en el contenido en energía metabolizable aparente corregida en nitrógeno (EMAn) entre diferentes tipos de trigo, con especial énfasis en la fracción de los hidratos de carbono (almidón (ALM) y polisacáridos no amiláceos (PNA)). Las dos principales razones para ello son, en primer lugar, que el trigo se utiliza fundamentalmente como fuente de energía, dado su elevado contenido en ALM y, en segundo lugar, que los PNA afectan negativamente su valor nutritivo, incluso a concentraciones bajas. La influencia de ALM en el contenido en EMAn del trigo depende de su cantidad y su digestibilidad, mientras que la influencia de los PNA depende, sobre todo, de la fracción soluble de PNA y de su capacidad para aumentar la viscosidad de la digesta en el intestino de los broilers. Los fabricantes de pienso han intentado controlar la variabilidad en el valor nutritivo del trigo midiendo el contenido de ALM en el momento de la recepción y, también, añadiendo enzimas degradadores de PNA al pienso. Los valores del coeficiente de digestibilidad del trigo suelen obtenerse de tablas de alimentación. Sin embargo, estas tablas sólo dan valores medios y no consideran la variabilidad entre los granos de trigo. Por tanto, los factores relacionados con la variación del valor nutritivo del trigo no están completamente resueltos y todavía necesitan investigación.

El objetivo de esta tesis ha sido estudiar los factores que influyen en el valor EMAn del trigo y su utilización por los pollos de carne. Para ello, se han realizado ensayos de digestibilidad y de crecimiento, utilizando la misma estirpe de animales. Los ensayos se realizaron con distintas muestras y variedades de trigo, cosechados en diferentes zonas geográficas o cultivados bajo diferentes condiciones. La variabilidad en la digestibilidad de ALM se estudió midiéndola a nivel fecal e ileal, así como la velocidad de degradación del mismo. Digestibilidades y AMEn se determinaron utilizando la técnica del marcador, con óxido de cromo(III). Los piensos utilizados contenían trigo como única fuente de almidón y harina de soja como la principal fuente de proteína, procurando utilizar composiciones de piensos lo más parecidas a las usadas en Europa. Todos los piensos satisfacían las necesidades nutritivas de los pollos en cada momento.

Se realizó una revisión bibliográfica antes de los ensayos (Capítulo 2), que se dividió en

el estudio de los factores físicos y químicos que influyen en el valor nutritivo del trigo. Al final, se recoge una breve descripción de la influencia de la edad del animal y su capacidad para digerir el trigo. Se concluye que el valor nutritivo del trigo no puede predecirse por los parámetros habitualmente analizados en el mismo. Además, se cuestiona la relación entre EMAn del trigo y el rendimiento de los pollos, así como el efecto de la adición de enzimas degradadores de PNA en igualar el valor nutritivo de distintas muestras de trigos. Se sugieren el contenido en proteína bruta (PB) del trigo, la velocidad de degradación de ALM y su sincronización con la digestión de PB como los principales factores que influyen en el valor nutritivo del trigo.

El Capítulo 3 estudia la variabilidad entre muestras de trigo de origen europeo. Se utilizaron 50 muestras de trigo procedentes de Francia, Irlanda, Portugal, España y Holanda, una vez aceptadas por el control de calidad de su correspondiente fábrica de piensos. En primer lugar, se analizaron sus propiedades físico-químicas en análogas condiciones de laboratorio y, posteriormente, cada muestra de trigo se incorporó en un pienso y se ofreció desde los 21 hasta los 30 días de edad a pollos alojados individualmente en jaulas. La energía metabolizable aparente corregida en nitrógeno y la retención de PB se midieron en las heces recogidas desde los 27 hasta los 30 días de edad, y la digestibilidad ileal de ALM y PB se midieron el día 30 de vida. Los resultados obtenidos mostraron que los trigos utilizados variaron principalmente en su viscosidad (CV = 30%) y PB (CV = 18%), mientras que los contenidos de ALM fueron más homogéneos (CV = 3.8%) de lo esperado. Las digestibilidades ileal y fecal de ALM resultaron elevadas (95.8%, de media) y parecidas en cuanto a variabilidad (CV = 2.4%). La variabilidad en EMAn fue del 5%. El contenido medio de EMAn del trigo fue un 7% inferior al de los valores reflejados en las tablas de alimentación. El contenido en PB afectó de forma negativa a la digestibilidad ileal de ALM y a su contenido en EMAn. El contenido en almidón fue el único factor que influyó de forma positiva en el valor EMAn de los trigos.

En el Capítulo 4 se estudió el efecto de la adición de enzimas degradadores de PNA sobre el valor nutritivo del trigo. La hipótesis fue que, aunque la adición de enzimas aumenta el valor nutritivo del trigo, esto no es suficiente para igualar el valor de diferentes muestras. Se utilizaron cuatro variedades de trigos y dos niveles de incorporación (0 y 1 g/kg de pienso) de una premezcla de enzimas reductores de la viscosidad en dos experimentos diferentes (crecimiento y metabolismo) coincidentes en el tiempo. En el primer experimento, se suministraron los piensos a pollos durante todo el periodo de cebo, y se midió la viscosidad intestinal durante 4 semanas consecutivas, comenzando el día 13 de vida. En el segundo experimento se midieron las digestibilidades de ALM y PB y el valor EMAn del pienso, durante los periodos de arranque y crecimiento. El efecto de los enzimas fue más evidente en los pollos jóvenes. La digestibilidad de ALM fue muy elevada a nivel ileal y fecal (98.53% de media).

La adición de enzimas reductores de la viscosidad no influyó en los resultados zootécnicos de los pollos, a pesar de que su empleo redujo la viscosidad intestinal y elevó el valor de EMAn un 1.1% durante el periodo de arranque. El efecto de la adición de enzimas sobre EMAn resultó depender de la variedad del trigo en el periodo de crecimiento, aumentando su valor en una de las cuatro muestras de trigo utilizadas. La utilización de de enzimas reductores de la viscosidad no eliminó las diferencias en el valor nutritivo de las variedades del trigo, al depender su efecto de la capacidad de cada trigo para aumentar la viscosidad.

La variación de la velocidad de degradación de ALM y su influencia en los resultados productivos de los pollos se midió en los Capítulos 5 y 6. En el Capítulo 5 se estudiaron dos factores: uno, la variación de la velocidad de degradación de ALM en distintas variedades de trigo y, dos, la influencia del nivel de abono nitrogenado en la velocidad de degradación de ALM. Las variedades de trigo se cultivaron en la misma área con dos niveles de abono nitrogenado. Se observaron diferencias en la velocidad de degradación de ALM entre las variedades de trigo (diferencia máxima, 25%), aún cuando la digestibilidad total de ALM fue similar entre variedades. Estas diferencias se debieron principalmente a las observadas en la parte superior del intestino delgado (yeyuno). El abonado nitrogenado no afectó a la velocidad de degradación de ALM.

En el Capítulo 6, la hipótesis fue que las variedades de trigo se podrían clasificar en base a su velocidad de degradación de ALM y que ésta podría influir en los rendimientos de los pollos. Para confirmarlo, se llevaron a cabo dos experimentos. En el primer experimento se utilizaron 3 variedades de trigo, una de ellas de dos orígenes distintos, para determinar la velocidad de degradación de ALM. En el segundo experimento, se formularon cinco piensos diferentes con el mismo contenido de ALM, utilizando tres variedades más una de ellas mezclada con guisantes. Se observó una diferencia máxima en la velocidad de degradación de ALM del 15% entre variedades, y del 23% entre la misma variedad con diferente origen. Los rendimientos de los pollos fueron afectados por la velocidad de degradación de ALM de forma linear y cuadrática. Los mejores resultados se obtuvieron con una velocidad de degradación próxima a 2.2 h^{-1}, siendo el efecto más evidente en el periodo de arranque que en el de crecimiento. Los niveles de glucosa en sangre se modificaron con el tiempo, pero no se observó un efecto del tratamiento, por lo que este parámetro no pudo utilizarse para predecir los rendimientos de los animales en función de la velocidad de degradación de ALM. Los conteos de Clostridium perfringens no se vieron afectados por el tratamiento, probablemente debido al bajo número de repeticiones utilizado. Sin embargo se observó una disminución, no significativa, en los conteos de Clostridium perfringens con la velocidad de degradación de ALM más baja.

Las principales conclusiones de estos estudios fueron las siguientes:

- No se puede predecir de forma precisa el valor energético del trigo a partir de sus propiedades físico-químicas. De todas las variables estudiadas, el contenido de almidón de los trigos fué el parámetro que mejor se relacionó.

- La digestibilidad ileal del almidón está negativamente relacionada con el contenido en proteína bruta del trigo.

- La variedad afecta al valor nutritivo y la utilización del trigo. El efecto de la adición de las enzimas utilizadas en este estudio sobre el contenido en energía metabolizable corregida en nitrógeno depende de la variedad y de la viscosidad de la digesta.

- El almidón de trigo se digiere principalmente en el yeyuno, sin que haya digestión posterior al íleon.

- La velocidad de degradación del almidón depende de la variedad de trigo y de algunos factores ambientales (ej. origen). El abono nitrogenado no influye en la velocidad de degradación del almidón.

- La velocidad de degradación del almidón influye en los rendimientos de los animales, especialmente en los más jóvenes.

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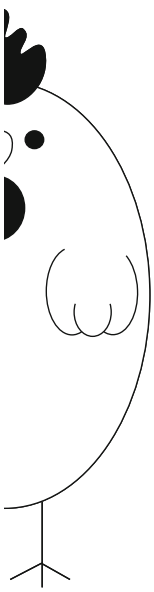

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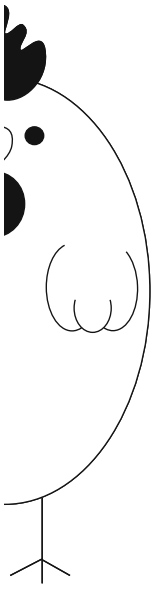

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Ángela (otra vez)

CURRICULUM VITAE

Ángela Gutiérrez del Álamo Oms nació en Bilbao (España) el 7 de Diciembre de 1972 en Bilbao, España. Se graduó en 1998 Cursó sus estudios en la Escuela Técnica Superior de Ingenieros Agrónomos de Madrid, dónde se especializó en producción y nutrición animal. Desde que se graduó trabaja en el departamento de investigación y desarrollo de Nutreco, primero como estudiante y posteriormente como investigadora en las áreas de materias primas y aditivos. Ha trabajado durante 3 años en Holanda, dónde Nutreco tiene el centro de experimentación porcina, y actualmente trabaja en España (Casarrubios del Monte), en el centro de experimentación de avicultura y cunicultura. Ángela ha compaginado su trabajo con su doctorado que trata sobre el valor nutricional del trigo en avicultura de carne. Doctorado dirigido por la E.T.S.I. Agrónomos de Madrid y la Universidad de Wageningen y cuyas pruebas se han realizado en la granja experimental de Nutreco en Casarrubios del Monte.

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