KINETICS OF STARCH DIGESTION

AND PERFORMANCE OF BROILER CHICKENS

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AND PERFORMANCE OF BROILER CHICKENS

R.E. Weurding

Proefschrift

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op gezag van de rector magnificus
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Stellingen

1. Kennis over de mate van zetmeelvertering bij vleeskuikens geeft onvoldoende informatie over de benutting ervan.
   Dit proefschrift.

2. *In vitro* systemen ter bepaling van verteringscoëfficiënten voor groeiende dieren dienen gekoppeld te zijn aan de leeftijd van de dieren.
   Dit proefschrift.

3. Retrogradatie van zetmeel treedt niet op tijdens de gangbare mengvoerproductie in Nederland.

4. In de toekomstige voederwaardering zal meer gebruik gemaakt moeten worden van *in vitro* systemen omdat voederwaarde meer is dan de som van de nutriënten.

5. De invloed van technologische behandelingen in de mengvoerfabriek op voederwaarde wordt nog steeds ondergewaardeerd.

6. Het creëren van genetisch gemodificeerde dieren is onverantwoord en zadelt toekomstige generaties met grote ecologische problemen op door verlies aan genetische diversiteit en uitschakeling van natuurlijke beschermingsmechanismen.

7. Een rundveerantsoen is als een voetbalelftal. De beste resultaten worden behaald als de spelers / grondstoffen met specifieke capaciteiten / nutriënten elkaar goed aanvullen en wanneer de linies / ruw- en krachtvoeders niet te ver uit elkaar liggen.

Stellingen behorende bij het proefschrift:
Kinetics of starch digestion and performance of broiler chickens.
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Abstract

Starch is stored in amyloplasts of various plants like cereals and legumes and seeds of these plants are used as feedstuffs for farm animals. Starch is the major energy source in broiler feeds. The properties of starch from different origin vary condiderably and these properties determine its resistance to enzymatic digestion. The objective of the research project described in this thesis was to study starch digestion behaviour and to investigate whether its digestion rate affects performance of broiler chickens. A digestion experiment pointed out that starch digestion of various native feedstuffs is not complete in four week old broiler chickens. Differences were observed in site, rate and extent of starch digestion. The major part of starch was digested in the upper small intestine (20 to 98%) and the amount of starch digested in the lower small intestine varied from 36 to 1%. Microbial fermentation of starch entering the hind gut did not occur. An in vitro method that simulates the digestive process in the broiler alimentary tract yielded digestion data that correlated well with in vivo starch digestion. Based on in vitro measurements, starch of various feedstuffs and diets could be divided into rapidly digestible starch, slowly digestible starch and resistant starch. These in vitro measurements pointed out that tapioca pellets were rapidly digestible, whereas native peas and sorghum were slowly digestible. Furthermore, technological treatments involving heat and moisture increased in vitro starch digestion rate considerably. Four growth experiments pointed out that feed efficiency of broiler chickens was better on diets with a slow starch digestion than on diets with a rapid starch digestion. Furthermore, the difference in feed efficiency between birds fed a rapidly digestible starch diet or a slowly digestible starch diet was bigger at low than at high protein levels. This suggests that protein efficiency of broiler chickens is higher on slowly digestible starch diets than on rapidly digestible starch diets. The interaction between starch digestion rate and protein level could not completely explain the difference in feed efficiency between birds on slowly- or rapidly digestible starch diets. Therefore, an improvement in energy efficiency may also be involved. In one experiment, feeding a slowly digestible starch diet resulted in a lower number of Clostridium perfringens bacteria in the caeca of broiler chickens than feeding a rapidly digestible starch diet. Therefore, starch digestion rate may affect the microbial balance in the broiler alimentary tract.

Keywords: starch, digestion rate, broiler chickens, peas, tapioca

Voorwoord

Ruim vijf jaar geleden werd ik door het Instituut voor de Veevoeding 'De Schothorst' benaderd met de vraag of ik interesse had om promotie onderzoek te doen naar de relatie tussen mengvoedertechnologie en de zetmeelvertering bij landbouwhuisdieren. Gezien het feit dat ik me gedurende mijn studie gespecialiseerd heb in de veevoeding met de nadruk op rundvee en mengvoedertechnologie was ik direct enthousiast. Het onderzoek diende echter naast rundvee ook betrekking te hebben op andere landbouwhuisdieren. Tot aan dat moment had ik zelfs nooit serieus nagedacht over promotie onderzoek. Dit is dan ook nooit mijn ambitie geweest. Maar het onderwerp, de toepassingsgerichtheid en de omgeving waarin het onderzoek uitgevoerd zou worden stimuleerden mij om er voor te gaan. Het duurde even voordat de lijn van het onderzoek er echt in kwam, maar toen die lijn er eenmaal was werd het enthousiasme alleen maar groter. Van de drie diersoorten die in het onderzoek zijn betrokken, koeien, varkens en vleeskuikens, werd de zetmeelvertering bij deze laatste diercategorie het meest uitgediept. Vandaar dat dit deel van het onderzoek in dit proefschrift is beschreven.
Vele mensen waren in meer of mindere mate betrokken bij dit onderzoek en ik heb op vele fronten steun en inspiratie van hen gekregen. Langs deze weg wil ik een aantal van hen bedanken voor hun bijdrage aan het in dit proefschrift beschreven onderzoek. Ik kon rekenen op de begeleiding vanuit twee hoeken. Dick Dijkshoorn, Bert Veldman en Wim Veen vanuit De Schothorst en Seerp Tamminga, Thomas van der Poel en Martin Verstegen vanuit de Leerstoelgroep Diervoeding van de Landbouwuniversiteit. Maar ook Piet van der Aar en René Kwakkel waren goede gesprekspartners in deze. Met name in de eerste anderhalf jaar is er veel werk verzet voor de ontwikkeling van een *in vitro* methode die de zetmeelvertering in het vleeskuiken kan voorspellen. Hiervoor heeft Arnold Dijkstra vele zetmeelanalyses uitgevoerd. Arnold en Henk den Hartigh hebben ook goed meegedacht in het ontwikkelingstraject. Toen de *in vitro* methode er een keer was is hij ook heel veel toegepast in het vervolg van het onderzoek. Saskia van Schuppen kan het weten, want zij heeft bijna alle *in vitro* bepalingen uitgevoerd. Op een gegeven moment stond een proef waar mijn naam bij stond gelijk aan veel zetmeelanalyses. Hoe eentonig de *in vitro* metingen wellicht waren, des te gezelliger waren de *in vivo* metingen. Tijdens de verteringsproeven was het altijd goed toeven in de sectie ruimte met de strippende dames van het laboratorium. Er zijn heel wat darmpjes in eerste instantie leeggestript en later leeggespoeld. Na de verteringsproeven kwamen de groeiproeven aan bod. Mijn proefopzetten waren vaak net iets anders dan volgens standaardprotocol en dat riep wel eens vraagtekens op bij collega's. Waarom moesten de kuikens ineens meelvoer vreten in plaats van korrels? Ook het voersysteem was hier niet op ingericht, waardoor het voorkwam dat we om beurten om de vier uur de stallen door moesten om aan alle voerbakken te draaien. De nachtrust was soms ver te zoeken. Ik was dan ook blij dat zowel de dierverzorgers

Wim Bronsvoort, Cees de Boer en Jack Morren als collega onderzoekers Henk Enting en Adriaan Smulders bereid waren in deze carrousel mee te draaien. Henk, Adriaan en Piet hebben ook een grote bijdrage geleverd bij de opzet en interpretatie van de groeiproeven.
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In de nazomer van 2001 moesten de laatste papers afgerond worden en dienden de afzonderlijke papers omgevormd te worden zodat er ze in dit proefschrift gebundeld konden worden. Marjolein Klingenberg heeft me enorm geholpen met de lay-out van het proefschrift. Ik dacht dat we dit binnen een week wel zouden klaren. Maar dat viel behoorlijk tegen. Marjolein, langs deze weg nogmaals bedankt voor je grote inzet.
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Aan mijn ouders

Contents

Chapter 1

General Introduction

Growing animals like broiler chickens require a daily amount of macro nutrients to fuel metabolism and to provide the precursors for synthesis of structural and functional macromolecules. These macro nutrients (carbohydrates, proteins and fats) are digested into smaller molecules in the gastrointestinal tract and they are subsequently absorbed. In feed evaluation systems, most attention is given to the energy- and protein value of a feedstuff. Energy and protein are necessary for functions associated with maintenance and for production. In addition certain micro nutrients like minerals and vitamins are also necessary for crucial processes in the body. Energy is not a chemically identifiable nutrient but is stored in organic compounds like carbohydrates, proteins and fats. Energy is a property that is manifested when these compounds are oxidised during metabolism. In birds, about 40% of the energy released during oxidation of nutrients is captured in the energy carrier ATP (Klasing, 1998). The energy captured in ATP is available for anabolic, catabolic, osmotic and mechanical work. It is important to supply the animals with a balanced diet. A balanced diet provides the birds with sufficient nutrients for both energy- and precursor supply. Protein is digested into peptides and amino acids, fat is digested into monoglycerides and free fatty acids and starch is digested into glucose. These digestion products are subsequently absorbed form the small intestine. They are partly utilised as precursors and partly used as energy sources. When a specific amino acid is oxidised for energy supply it is lost for protein synthesis.

Starch is an important energy source for farm animals in general and broiler chickens in particular. More than 50% of the energy requirements of today's broiler chickens is met by starch in the diet. Starch is completely built up of glucose molecules and glucose is a key metabolite in the intermediary metabolism of animals. Another positive feature of starch is the fact that it is relatively easy accessible for enzymatic digestion, because the glucose molecules are linked through α-bonds. Animals produce enzymes which have the ability to cleave these type of bonds. Cellulose, another polymer that is completely composed of glucose molecules, is inaccessible for enzymatic digestion in the small intestine because the glucose molecules are linked through β-bonds, which cannot be hydrolysed by enzymes secreted by the animal.

Starch is found in various plants and serves as a storage carbohydrate. It is generally found in seeds of cereal grains and legumes, but also in roots (cassava) and tubers (potatoes). Native starch (i.e. unprocessed starch) is stored in granules. These bodies are variable in size and shape. Starch granules contain two different glucose polymers: amylose and amylopectin. Amylose is a linear polymer and amylopectin is a highly branched polymer that gives native starch its crystallinity. The amylose to amylopectin ratio in the starch granules is also variable. Finally, there are three types of amylopectin structure in native starch and these types differ in susceptibility to enzymatic hydrolysis. Starch structures can be modified by mechanical and (hydro) thermal processing.

The kinetics of starch digestion in farm animals depends on many factors. The accessibility of starch to digestive enzymes in the small intestine is probably the most important factor. The physicochemical structure of the seeds greatly determine the degree of accessibility of starch for enzymatic hydrolysis. However, the composition and structure of the starch fraction itself also affect the degree of accessibility of starch. These characteristics vary between plants species and also depend on growing conditions (geographic area, weather and soil conditions).

The primary objective of commercial broiler farms is to establish a rapid and efficient growth of the broiler chickens. This can be achieved by feeding diets that contain readily digestible nutrients. In order to be utilised by broiler chickens, starch should be digested to glucose by amylolytic enzymes in the small intestine or fermented into volatile fatty acids by the microflora residing in the caeca or colon. Starch digestion in the small intestine yields more net energy than fermentation of starch in the hind gut (Dierick, et al., 1984). For an efficient growth of the broiler chickens most dietary starch should be digested in the small intestine. It is generally believed that in most species starch digestion is almost complete at the end of the small intestine. Therefore, starch from different origin is regarded as the same entity and assumed to be interchangeable in feed evaluation systems. Differences in starch characteristics that exist between starch sources are not accounted for. This in spite of the fact that we know that accessibility of starch varies considerably between feedstuffs. Although differences in total extent of starch digestion between most feedstuffs are probably small, differences in the site and rate of starch digestion may be more pronounced. This is visualised in Figure 1, in which two examples are shown of starch digestion as a function of time during which a feedstuff is exposed to enzymatic digestion.

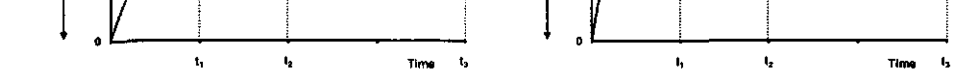

Figure 1. Example of different starch digestion rates. Figure a represents a slow starch digestion and Figure b represents a rapid starch digestion. Starch digestion rate is related to the site of starch digestion, assuming a constant passage rate. SI_1 = first part of small intestine; SI_2 = second part of small intestine and SI_3 = third part of small intestine.

Figure 1a represents a slow starch digestion and Figure 1b represents a rapid starch digestion. In both figures, enzymatic starch digestibility is similar (96%). Total starch digestion of the rapidly digestible starch source has already been reached at t_2, whereas the slowly digestible starch source required considerably more time (t_3) to reach this extent of starch digestion. The small intestine can be divided into different segments and when passage rate through the small intestine is not affected by the type of diet, exposure time corresponds to a specific site of the small intestine (SI_1, SI_2 and SI_3). From Figure 1 it is clear that a slow starch digestion results in more starch digestion in the posterior part (SI_2 and SI_3) of the small intestine than a rapid starch digestion (28 and 6% respectively).

Efficiency of starch utilisation may be dependent on the site or rate of starch digestion. Therefore, it is important to know whether diets with the same amount of digestible starch, but different rates of starch digestion, affect performance of growing broiler chickens. If performance of broiler chickens is indeed affected by starch digestion rate, this property must be measurable. Therefore, it is important to develop an *in vitro* assay that can predict starch digestion rate in the small intestine of broiler chickens.

The outline of this thesis is as follows (Figure 2).

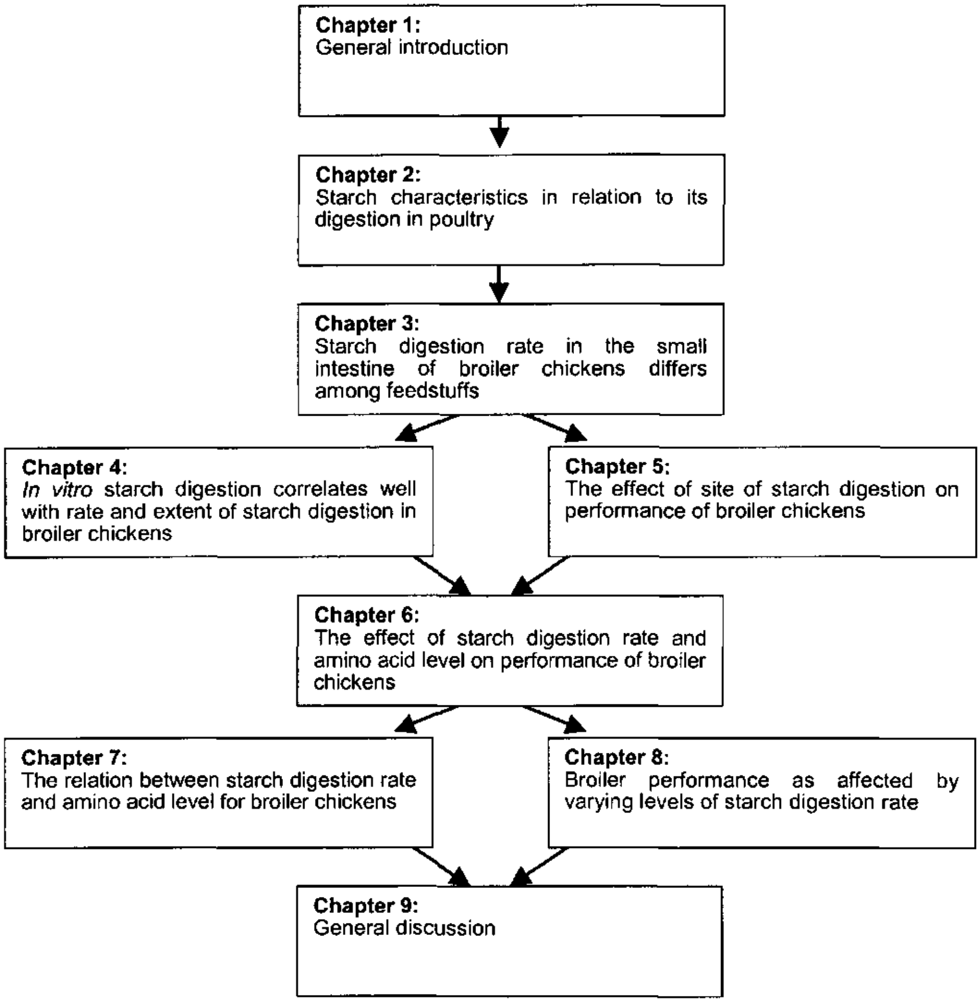

Figure 2. A schematical representation of the outline of the thesis.

First, a description of starch, its properties and its place in a feedstuff is given. These characteristics are related to starch digestion in poultry (Chapter 2). For the first experiment, twelve starch rich feedstuffs were selected based on differences described in this literature review and availability. Starch digestion along the small intestine and in the hind gut of broiler chickens was measured for these twelve feedstuffs (Chapter 3). In this digestion trial, differences in site, rate and extent of starch digestion were determined. This experiment yielded important data for the following phases in the project. The starch digestion coefficients of the feedstuffs at the various sites of the small intestine served as reference values for the *in vitro* assay that was being developed.

The same diets as used in the *in vivo* digestion trials were subjected to an *in vitro* system that simulates the digestive process in the broiler chicken (Chapter 4). This *in vitro* method can be used to predict starch digestion rate in feeds. Furthermore, differences in starch digestion rate between feedstuffs and between batches of feedstuffs can be determined. The *in vitro* system can also be applied to study the effects of processing on starch digestion rate. The starch digestion coefficients from the *in vivo* trial (Chapter 3) were used to formulate broiler diets with different starch digestion coefficients at the posterior jejunum. The starch sources used in the first growth trial with broiler chickens were from the same batch as used in the digestion trial. The objective of this growth trial was to investigate whether differences in site of starch digestion affect broiler performance (Chapter 5).

When the *in vitro* method was ready to be used, the effect of processing on starch digestion rate was studied using the *in vitro* test. Based on the results of the *in vitro* measurements and the obtained knowledge of starch digestion rate of different feedstuffs a second growth trial was carried out. The primary objective of this experiment was to confirm the results from the previous growth trial. In this second growth trial, differences in starch digestion rate were established by using different starch sources and by means of processing of starch sources (Chapter 6). The advantage of using processing of starch-rich feedstuffs over diversity of starch sources is the fact that in the former case the other ingredients are exactly the same. Another objective of the experiment described in Chapter 6 was to investigate whether starch digestion rate has an effect on amino acid utilisation. This was further investigated in Chapter 7. Finally in Chapter 8, an experiment is described in which the effect of various levels of starch digestion rate on broiler performance was studied.

Chapter 2

Starch Characteristics in Relation to its Digestion in Poultry

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Abstract

Starch is an important energy provider for most farm animals. Differences in starch characteristics do exist among feedstuffs. Starch is composed of two glucose polymers: amylose and amylopectin. Amylopectin gives the native starch granule its crystallinity by means of hydrogen bonds and van der Waal's forces. Amylopectin from different starch sources show different starch structures with varying stability: A, B and C-type starch. The amylose to amylopectin ratio also differs amongst feedstuffs. Other discriminating starch characteristics are granule size and side chain length. When starch is heated in the presence of sufficient water, starch starts to gelatinise. This is a term indicating the loss of crystallinity of the starch granule. In some circumstances, starch can recrystallise after cooling. This process is termed retrogradation. In retrograded starch, the crystallinity is mainly caused by amylose. In the gastrointestinal tract starch is hydrolysed to α-limit dextrins, maltotriose and maltose by α-amylase which is secreted in the intestinal lumen. These products are further hydrolysed to glucose by enzymes in the intestinal wall and glucose is subsequently absorbed from the small intestine. A variable resistant starch fraction escapes absorption and enters the hind gut. Only a limited fraction of resistant starch is fermented to volatile fatty acids by the microflora residing in the hind gut. Starch digestion in poultry is determined by the digestive capacity of the bird. Young birds have a lower digestive capacity than older birds. However, the major factor affecting starch digestion is the accessibility of the starch fraction for digestive enzymes. Accessibility is determined by several factors like viscosity of the gut contents, protective structures surrounding the starch granules, particle size and starch characteristics. Digestion trials with poultry indicate that for most feedstuffs starch digestion is high, but not always complete. Starch digestion in poultry is not the same for all feedstuffs. Processing of feedstuffs with a poor starch digestion can improve starch digestion considerably.

Keywords: starch, digestion, poultry, processing

Introduction

Starch is the main component of important feedstuffs like cereal grains, peas, beans and tapioca. Poultry feeds contain considerable amounts of these starch sources. For instance, typical starch contents of diets for broilers and layers are 36 and 34% respectively. Starch, which is in general readily digested, is the major energy supplier for these animals. More than 50% of the metabolisable energy in poultry feeds is provided by starch. The site of the alimentary tract where the major part of starch digestion takes place differs between starch sources. Starch utilisation is most efficient when the starch is digested in the small intestine because in this part of the gastrointestinal tract starch is broken down to glucose. Glucose is subsequently absorbed by the intestinal wall. Starch digestion in the hind gut is carried out by the micro-flora and they ferment glucose to volatile fatty acids (VFA), methane, hydrogen and carbondioxide. VFA make up 90% of these fermentation products. Energy is lost with the other products and also in the form of fermentation heat. Furthermore, the efficiency of the utilisation of VFA in the intermediary metabolism of the animal is lower than the utilisation of glucose as such (Dierick et al., 1984). Processing, involving heat, moisture and shear forces, generally reduces particle size and changes the crystalline structure of the starch. These effects make starch more accessible for digestive enzymes. Therefore, starch will be digested more rapidly and this will lead to shifts in the site of starch digestion. Considerable differences exist in the rate of starch digestion and the extent of starch digestion at the end of the ileum. In order to understand the differences in starch digestion it is necessary to know which factors affect starch digestion. Conditions in the gastrointestinal tract of the animal, physico-chemical properties of the starch containing feedstuff and starch characteristics are factors that should be considered. In this review, the structure and composition of starch is described first. This is followed by a brief description of the changes in starch structure during gelatinisation/melting and retrogradation. Then, differences in several characteristics of various starch sources are indicated. Subsequently, the process of starch digestion is described and an overview of starch digestibility values is given for poultry. It is tried to link the differences in starch digestibility in poultry to the differences in the feedstuffs and starch characteristics as described in the first parts. In this paper the terms digestion, degradation and breakdown are regarded as synonyms, and the term hydrolysis is used specifically when chemical bonds are broken enzymatically.

Composition and structure of the starch granules

Starch, a storage carbohydrate, is a mixture of two different glucose polymers: amylose and amylopectin (Figure 1). These two molecules form the major part of the starch granule, a body in which the starch is found in the plant. Amylose is an almost linear polymer build up by α-1,4 bound glucose units. Amylose has only 9 to 20 branches in its molecule with chain lengths varying from 4 to over 100 glucose units (Oates, 1997). The molecular weight varies from 50,000 to 1,000,000 Dalton (Hoseney, 1986; Heijnen, 1997; Oates, 1997). Amylopectin is a branched polymer which clusters many short linear α-linked glucose chains by linking them with α-1,6 bonds. The α-1,6 bonds make up approximately 5% of the total glycosidic bonds in amylopectin (Gallant et al., 1992). This means that the average glucose chain in amylopectin is made up of 20 glucose units. The range of molecular weights reported for amylopectin vary from one million to one billion Dalton (Hoseney, 1986; Heijnen, 1997; Oates, 1997).

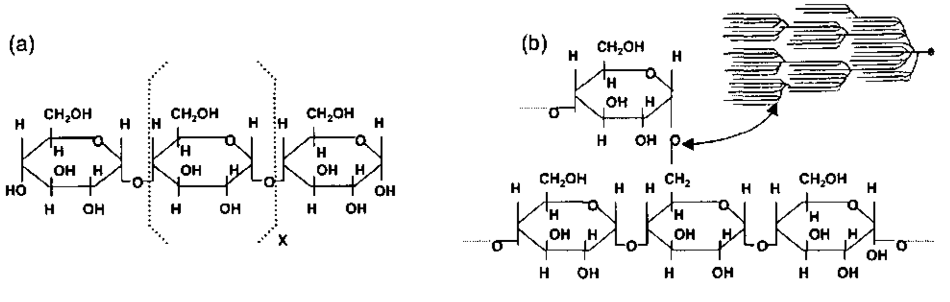

Figure 1. Chemical structure of (a) amylose and (b) amylopectin (taken from Zobel, 1988).

Starch granules are found in the amyloplasts of the plant. The starch granule grows concentrically, starting at a site called the hilum. The hilum is the centre of the starch granule and this area is usually less organised than the rest of the granule (Keetels, 1995). Most of the reducing ends of the starch molecules are located here (Oates, 1997). During growth the amylopectin chains are extended simultaneously (Rooney and Pflugfelder, 1986). The energy for growth is supplied by the sun via photosynthesis. Granule size varies from 2 to 100 μm (Keetels, 1995) and depends on the plant species and the stage of development the plant is in. Granules in tubers are generally bigger than those in cereal grains. The native starch granule contains a large number of macro-molecular chains which are organised in crystalline structures. The crystalline structure refers to the double helix formations, which is a result of the intertwining of glucose chains within the amylopectin molecules (Oates, 1997). It is generally accepted that amylopectin is responsible for the crystalline structure of the starch granule (Imberty et al., 1991). In fact, starch granules can be made from amylopectin alone, as is the case in some mutant varieties without amylose (waxy maize). The short branched chains in amylopectin form the local organisations in the molecule. These chains form rigid double helices which are bound in pairs and stabilised by hydrogen-bonds and van der Waals' forces. There are no hydrogen bonds within the chains, but they are found between the two helices and are responsible for approximately 40% of the stabilisation energy of the double helix. The remaining stabilisation energy comes from van der Waals' forces (Imberty et al., 1991). Hydrogen bonds are also formed between the double helices (both directly and indirectly through the water molecules).

By use of X-ray diffractometry, the structure of the crystalline amylopectin can be determined. Three forms of crystallisation can be distinguished in the native starch granule: the A- and B type are the most extreme forms and C type starch is an intermediate form. The double helix in A-type starch is very compact and there is no space left for water or any other molecule in its centre (Imberty et al., 1991). In B-type starch the double helices are connected through a network of hydrogen bonds which form a channel inside the hexagonal arrangement of six double helices. The channel is filled with water molecules, half of which are bound to the linear chains of amylopectin and the other half to the other water molecules (Gallant et al., 1992). The density of the crystallites is less and the water content is higher in B-type starch than in A-type starch. There is controversy about the C-type starch, it could be a distinct structure, but it could also be a mixture of A- and B-type granules. Perhaps all the starch granules have both A- and B-type structural patterns.

In general, starch in cereal grains and tapioca (Jane et al., 1997) is A-type starch (with the exception of high amylose starch varieties), tubers and high amylose cereal varieties have B-type starch and most legumes contain C-type starch (Gallant, 1992; Eliasson and Gudmundsson, 1996). The starch type of tapioca is not clear. According to Jane et al. (1997) tapioca starch is of the A-type; however, Oates (1997) and Zobel (1988) consider tapioca starch as C-type starch. A so called V-type starch can be found in gelatinised lipid containing starch (Eliasson and Gudmundsson, 1996). Retrograded starch shows the B-type pattern. Starch from wheat and rye has been reported to contain low levels of B-crystallites (Eliasson and Gudmundsson, 1996), which makes the structure even more complex. The formation of A or B starch depends on the chain length and the water content in the starch granule.

Several researchers found that the linear chains in amylopectin do not crystallise if these chains contain less than 10 glucose units (degree of polymerisation (DP)<10); the A-type results from chains with DP 10 to 12 and the B-type crystallites are found when DP>12. When the chains are longer than 50 glucose units, they do not form single crystals like the shorter chains do, but complicated networks are formed (Imberty et al., 1991). However, Hoover (1995) and Jane et al. (1997) refer to A, B and C-type starch formation when average chain lengths are 23-29, 30-44 and 26-29 respectively. It appears that the formation of a certain starch type is controlled by both the linear chain length and the extent of hydration. This can be explained by comparing the ways by which A- and B-type starch are formed. The linear chains in amylopectin can associate with each other as long as their DP is high enough. Two double helices are paired to form the stable duplex. If sufficient water is available to fill the central cavity and if the duplexes are long enough to organise these water molecules in a stable column, B-type starch will be formed. If not, then A-type starch will be formed.

Amylose and the branching areas in amylopectin form the amorphous regions in the starch granule. Crystalline and amorphous zones alternate within the starch granule (Jenkins et al., 1993; Imberty et al., 1991). When the starch granules are examined by electron microscopy, pronounced concentric rings become visible. The alternating crystalline and amorphous zones form the semi-crystalline growth rings and amorphous rings are found in between. Thus, there are three structural types for the organisation of crystalline and amorphous areas: crystalline domains, amorphous areas with branching points that alternate with the crystalline domains, and a second amorphous phase, which surrounds the alternating crystalline and amorphous areas (see Figure 2). The existence of growth rings suggests that the starch granules grow radially. Glucose units can be added at the non-reducing ends of amylose and amylopectin, located at the surface of the starch granule (Oates, 1997).

It is obvious that starch is a very complex substance. Its precise structure has not been revealed yet. It is not clear how all the information we have fits to form the complete starch granule. The location and role of amylose molecules within the starch granule is not known yet.

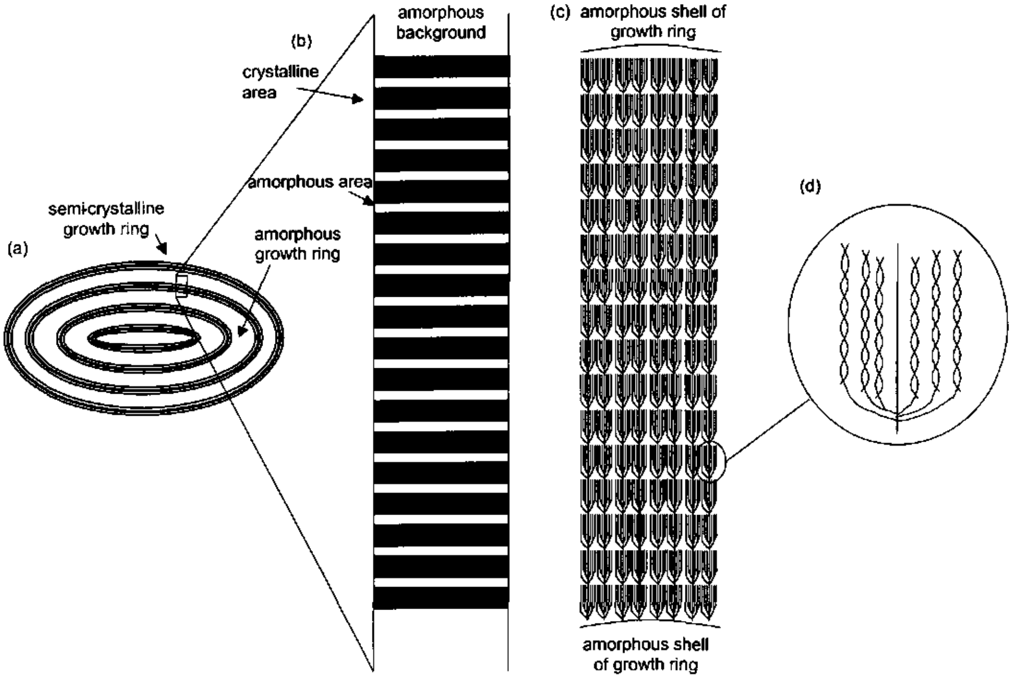

Figure 2. Schematic presentation of the structure of a starch granule. (a), Starch granule showing semi-crystalline concentric rings; (b), each ring contains stacks of amorphous and crystalline lamellae. (c), The cluster arrangement of amylopectin, the branching points form the amorphous region and the intertwining side chains form the crystalline regions as can be seen in (d). (Taken from Jenkins et al., 1993).

Starch gelatinisation and retrogradation

Starch gelatinisation

Starch gelatinisation is a collection term for describing a number of irreversible processes which occur when starch is heated in the presence of water. The temperature that is necessary for these processes to occur is called gelatinisation temperature. At temperatures when starch gelatinisation has not started yet (below gelatinisation temperature), native starch is insoluble in water due to the semi-crystalline structure of the starch granule and the hydrogen bonds which exist between the helices. Individually these bonding forces are relatively weak, but together they are strong enough to prevent starch from dissolving in cold water. When starch is heated above the gelatinisation temperature with an excess of water, the granule structure will be irreversibly altered. The starch granules absorb water and begin to swell, which leads to an increase in viscosity. At further heating the starch granule starts to loose its shape and soluble starch (especially amylose) is released to the solution. Hydrogen bonds between the linear segments in the crystalline regions, and to a lesser extent between the molecules in the amorphous regions, are broken and free polymers are dissolved. The soluble starch and the ongoing water uptake by the remnants of the starch granules cause a further increase in viscosity. During cooling the free polymers can form a network. These changes that occur after gelatinisation are referred to as gelation. It should be noted that the gelatinisation temperature always concerns a temperature range. This range is 1 to 2°C for an individual starch granule and the range for the whole population of starch granules in one starch source is usually between 5 and 15°C. The gelatinisation temperature range varies among different starch sources. Parameters that are often used to show the gelatinisation temperature range are T_0 (T at which gelatinisation starts) and T_e (T at which the gelatinisation process has finished).

The gelatinisation process starts in the amorphous regions, penetration of heat and moisture in the crystalline areas is slower. The swelling of the amorphous regions causes stress which helps to break the crystalline regions. The gelatinisation temperature depends on the amount of available water. Water softens the amorphous regions and supplies the crystalline regions with sufficient mobility to melt (Rooney and Pflugfelder, 1986). If water is the limiting factor, as is often the case in compound feed, more heat or mechanical energy is needed to soften the amorphous regions and to break the crystallinity (Rooney and Pflugfelder, 1986). At low moisture contents the gelatinisation process is often referred to as melting: the disappearance of the native starch crystallinity at low hydration levels. During this process the starch granule does not necessarily disappear. There is no clear boundary between melting and gelatinisation, the melting temperature is a function of the water to starch ratio (Figure 3). The melting temperature can increase to 150°C when the water to starch ratio decreases.

There are several ways to measure the degree of gelatinisation: measuring loss of birefringeance, differential scanning calorimetry (DSC), X-ray diffractometry and enzymatic methods. These methods measure the disorganisation of the crystalline domains one way or another. The granules that are left over after gelatinisation (ghosts) mainly contain amylopectin without a crystalline order. The structure of gelatinised starch is of the V-type.

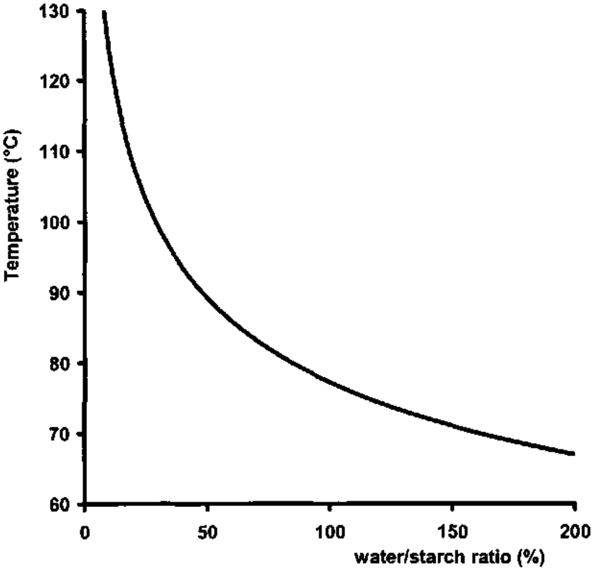

Figure 3. Example of gelatinisation temperature starch as a function of water content (% of starch) for a specific statrch. (Taken from Colonna and Champ, 1990).

Retrogradation

The transition from order to disorder that has taken place during gelatinisation or melting can be reversed after cooling the starch. During starch retrogradation the amylose (and to a lesser extent amylopectin) chains are linked together resulting in a more organised structure. Technically, starch retrogradation is not exactly the reverse process of gelatinisation. In order to understand the process of starch retrogradation, the whole picture, from native starch, via gelatinised starch, to retrograded starch has to be regarded. During gelatinisation intact starch granules swell because of water uptake. At one point a rigid substance will occur in which the big granules cannot move freely anymore. This substance is not a gel however. During further gelatinisation hydrogen bonds are disrupted and free polymers dissolve. During cooling of the substance the free polymers can form a network and at this point a gel is formed. No granular structure can be seen anymore. During retrogradation the water is pushed out of the gel and the starch precipitates. Retrogradation is in fact the return from a dissolved, disperse, amorphous stage to an insoluble, aggregated or crystalline stage (Swinkels, 1985). In native starch, amylopectin is responsible for the crystalline structure; in retrograded starch the crystalline structure is mainly caused by amylose. The chain length of the amylose molecules is an important factor and determines how easy starch can be retrograded. There is an optimal chain length for retrogradation. Retrogradation of amylose can occur within minutes to some hours, while retrogradation of amylopectin may take several weeks. This is due to the shorter linear side chains in amylopectin, which are responsible for the crystallinity. However, Fisher and Thompson (1997) found that amylopectin in heated maize starch (30% starch solution) showed retrogradation symptoms after just one day. They also indicated that the thermal history (final temperature used during gelatinisation) affects the extent of starch retrogradation, even when the starch source has been heated well above the gelatinisation temperature.

As mentioned before, starch gelatinisation and retrogradation are processes that occur when an excess of water is present. In the compound feed industry the thermal processes that are being used involve much less water and therefore the term melting is more appropriate. It is not known whether retrogradation takes place during feed processing in the feed plant.

Differences in starch characteristics between feedstuffs

In human and animal nutrition, several starch sources with varying starch characteristics are used. These differences can refer to the composition and structure of the starch granules and / or of the feedstuff itself. The starch granules may be very susceptible to enzymatic breakdown, but if the granules are embedded by some sort of a protecting layer, then the granules are still inaccessible for the enzymes. According to Moran (1982) starch degradability is a function of granule surface area, starch structure and degree of crystallinity. The differences that can be found between various starch sources are outlined in this section. It is likely that the differences in starch properties are partly responsible for differences in starch digestion in poultry.

Granule size

Franco et al. (1992) investigated the effect of granule size on *in vitro* starch hydrolysis rate in cassava- and maize starch. Granule size classes were: small (< 10 µm), intermediate (between 10 and 15 µm) and large (> 16 µm). They found that small granules were hydrolysed more rapidly than large granules (especially in the cassava starches). Microscopic observations indicated different mode of enzyme actions in small and large granules. The authors suggested that starch structure might be different in granules of different size. Small granules also showed higher initial gelatinisation temperatures and lower amylose contents for both starch sources. Small granules from cassava possessed less crystalline regions than large granules, which can explain the higher rate of starch hydrolysis in these granules. Effect of granule size on crystallinity was not given for maize starch. In literature the range for granule sizes are given for different starch sources. These ranges are very wide (Table 1). Starch granules from rice, tapioca, sorghum, maize and oats are small to intermediate, while starch granules from peas, faba beans and potatoes are relatively big (Cone and Wolters, 1991).

Table 1. Granule size and shape of different starch sources.

Starch source	Type	Granule size (diameter, µm) range	Average	Shape	Source
Maize	cereal	3-26	15	round, polygonal	1
Potato	tuber	5-100	33	oval, spherical	1
Wheat	cereal	2-10	15	lenticular	1,3
		20-35		round	3
Tapioca	tuber	4-35	20	oval, truncated	1
Sorghum	cereal	3-26	15	round, polygonal	1
Rice	cereal	3-8	5	polygonal, angular	1
Barley	cereal	20-25		round	3
		2-6		lenticular	3
Triticale	cereal	19	15	round	3
Rye	cereal	28	12	round, lenticular	3
Oats	cereal	3-10		polyhedral	3
Waxy maize	cereal	3-26		round, polygonal	1
Amylomaize	cereal	3-24		round, deformed	1
Mung bean	legume	10-32		oval, irregelar, round	2
Faba bean	legume	20-48		oval, spherical	2
Smooth pea	legume	20-40		oval, round	2
Wrinkled pea	legume	6-80		round	2

1) Swinkels, 1985; 2) Hoover en Sosulski, 1991; 3) Hoseney, 1986.

Amylose content

Starch in conventional starch sources contains 17 to 33% amylose. Amylose content is related to the growth stage of the plant. The amylose proportion in the granule as well as the molecular size of amylose and amylopectin increases during growth of the granule (Swinkels, 1985). This might explain some of the results found by Franco et al. (1992). Genetically modified grains like barley, maize, rice and sorghum contain waxy starch types and high-amylose starch types. Waxy starch contains only amylopectin (hardly any amylose). Amylose content in starch granules of high amylose maize varieties may be as high as 50 to 70%. The conventional starch sources can roughly be divided in three classes with high, intermediate and low amylose contents. Legume starches contain on average 33% amylose, cereal starches 25 % and tubers are relatively low in amylose (potato 20% and tapioca 17%). Rice is a cereal grain that is discerned from the other cereals with respect to amylose content of starch (17%).

High amylose starches are often referred to as less digestible. Amylopectin is a much larger molecule than amylose and therefore the surface area per molecule is substantially larger. Additionally the hydrogen bonds linking the glucose chains in amylose starch make the amylose molecules less susceptible to amylase attack compared to the amylopectin molecules, since the linear chains in the latter are much shorter. Amylose can easily form complexes with chemical compounds like lipids (fatty acids). Stable complexes as formed by amylose have not been reported for amylopectin. This is probably due to the shorter chain length and disposition of the linear segments of amylopectin (Zobel, 1988). When starch is subjected to heat treatments, amylose content plays an important role with regard to starch retrogradation, as was illustrated by an experiment described by Sievert and Pomeranz (1989). They found a positive correlation between amylose content and the formation of starch that is resistant to *in vitro* digestion (resistant starch). The amylose content and resistant starch fraction of several starch sources are given in Table 2.

Table 2. Amylose content and yield of *in vitro* resistant starch fraction after autoclaving different starch sources.

Starch source	Amylose content (%)	Resistant starch (%, DM basis)
Amylomaize VII	70	21.3
Amylomaize V	53	17.8
Pea	33	10.5
Wheat	25	7.8
Maize	26	7.0
Potato	20	4.4
Waxy maize	<1	2.5

Source: Sievert and Pomeranz (1989).

Zobel (1988) reported degrees of crystallinity for several starch sources (Table 3). No clear relation between amylose content and crystallinity was found, although the extreme starch sources (with regard to amylose contents) seem to indicate that a low amylose content can result in a higher degree of crystallinity. Since the crystallinity in the starch granules comes from amylopectin, it is logical that higher amylose contents result in less crystallinity. In their review about the physicochemical and functional aspects of starch, Eliasson and Gudmundsson (1996) confirm this relation. The fact that starch sources with different amylose contents in starch can have a similar degree of crystallinity suggests differences in amylopectin structure (chain length, branching pattern).

Length of amylopectin side chains

Chain length of amylopectin side chains vary according to the starch source. As mentioned before, chain length is an important factor determining whether A, B or C-type starch develops. The rate of starch retrogradation is presumably also affected by chain length (Hoover, 1995). Table 4 shows average values for amylopectin side chain lengths and is partly taken from Hoover (1995).

Table 3. Crystallinity and amylose content of different starch sources.

Starch source	Crystallinity %	Amylose %
	A-type starch	
Oats	33	23
Rye	34	26
Wheat	36	23
Sorghum	37	25
Rice.	38	17
Maize	40	27
Waxy maize	40	0
	B-type starch	
Amylomaize	15-22	55-75
Potato	28	22
	C-type starch	
Tapioca	38	18

Source: Zobel (1988).

Composition and structure of the feedstuff

Ruminant trials have shown that starch degradability in the rumen of e.g. dairy cows varies considerably between feedstuffs. These differences cannot solely be attributed to differences in the structure and chemical composition of the starch granules itself. For instance, starch of most cereal grains has an average amylose content of 25% (with the exception of genetically modified varieties) and the starch structure of these cereals is from the A-type. However, starch degradation rate in the rumen differs between cereal grains. The endosperm structure in which the starch granules are embedded as well as the composition of the cell walls surrounding the endosperm cells may be an important factor affecting rate of starch degradation in the rumen. The so called protein matrix that surrounds the granules as well as the Non Starch Polysaccharide (NSP) fraction in the kernels affect the accessibility of the starch granules to enzymes.

Table 4. Average side chain length of amylopectin from different starch sources (units / chain).

Starch source	Chain length
Maize	28
Waxy maize	19-24
Amylomaize	44
Rice	25-28
Wheat	17-25
Tapioca	26
Potato	34
Smooth pea	22
Faba bean	21

Source: Hoover (1995).

The cereal grain is roughly composed of the protecting pericarp, the germ and the endosperm. The pericarp and the germ contain hardly any starch and form only a small fraction of the grain (Kotarski et al., 1992). The endosperm accounts for about 80% of the total kernel weight. The cell walls of endosperm cells surround starch granules embedded in a protein matrix (McAllister and Cheng, 1996). Most starch is present in the endosperm which in some cereals can be subdivided in the aleurone layer, the peripheral endosperm, the horny endosperm and the floury endosperm. The aleurone layer contains no starch, but protein- and lipid bodies can be found here (Kotarski et al., 1992). Barley and wheat endosperm is very homogenous and the starch granules found in the endosperm are loosely associated with the protein matrix. The endosperm of maize and sorghum, however, comprises two distinct regions, the horny and the floury endosperm. The association between starch and protein in the floury endosperm is similar to that in the endosperm of barley and wheat. In the horny endosperm the starch granules are tightly embedded in the protein matrix (Hoseney, 1986). The proportions of peripheral, horny and floury endosperm vary among cereal grains (Kotarski et al., 1992).

The waxy genotypes of various cereal grains do not only show a much lower amylose content (close to zero) than normal varieties, but are also more susceptible to amylase attack. This is mainly due to differences in the peripheral endosperm structure (less peripheral endosperm, bigger starch granules and more equally distributed protein storage bodies). Michalet-Doreau and Champion (1995) found that starch in floury maize varieties was more degradable than starch in vitreous (horny) maize varieties. The protecting role of the protein matrix, which surrounds the starch granules, was very clear.

As mentioned before, starch is located inside the cells in the endosperm of cereal grains. These cells are surrounded by complex cell walls, which are composed of several types of carbohydrates. Cellulose is only found in small quantities in these cell walls and does not affect the starch degradation to a great extent. The NSP fraction (including ß-glucans and arabinoxylans) can affect starch degradation in monogastric animals. These materials are present in most cereal grains but the total amount and the proportion varies considerably among cereal grains (Classen, 1996). Cell walls in the endosperm of barley and oats contain considerable amounts of ß-glucans. Wheat, rye and triticale contain considerable amounts of arabinoxylans. Beta-glucans and arabinoxylans may serve as physical barriers in the digestive tract and can also increase the viscosity of gut contents. These factors can have detrimental effects on starch digestibility in monogastric animals.

Gelatinisation characteristics

Granule size, amylose content and degree of crystallinity are properties that are present in native starch. Another way of comparing different starch sources is by studying the starch behaviour during heat treatments. The combination of heat and moisture can cause starch gelatinisation. During gelatinisation, the starch granules loose their polarisation- or Maltese crosses, which is referred to as loss of birefringeance. The Maltese cross starts to fade at the hilum and this rapidly extents to the periphery (Swinkels, 1995). The loss of the Maltese cross indicates loss of crystallinity. Measuring this phenomenon can be done by using the Kofler hot-stage microscope. When a starch suspension is heated, some granules will start to gelatinise at a certain temperature. However, as mentioned earlier, starch gelatinisation temperatures always imply temperature ranges. Other granules start to gelatinise at higher temperatures. The Kofler gelatinisation temperatures are shown in Table 5. The given temperatures correspond to the loss of birefringeance by 5 (T_0) and 95% (T_e) of the granules. The gelatinisation temperature range of barley, oats and triticale are at a relatively low level and the range of sorghum, rice and amylomaize are at a relatively high level. Amylomaize shows a wide temperature range (25°C), while the faba bean and pea show a very narrow range. A feedstuff with a wide temperature range probably contains more variable starch granules than a feedstuff with a narrow temperature range. The onset and final gelatinisation temperature of a feedstuff can vary among varieties and because of different measuring conditions (water content of the starch solution).

Table 5. Starch gelatinisation temperature ranges (°C) of various starch sources.

Starch source	Onset temperature* (T_0)	Final temperature** (T_e)	Source
Wheat	58	64	1,2
Barley	51	60	2
Oats	53	59	2
Rye	57	70	2
Maize	62	72	1,2
Sorghum	68	78	1,2
Rice	68	78	1,2
Triticale	55	62	2
Waxy maize	63	72	1,2
Amylomaize	67	92	1
Potato	58	68	1
Tapioca	59	69	1
Horse bean	61	70	3
Faba bean	61	66	3
Pea	65	69	3

1) Swinkels (1985); 2) Hoseney (1986); 3) Hoover and Sosulski (1991).
* Onset temperature: temperature corresponding to the loss of birefringeance by 5% of the starch granules.
** Final temperature: temperature corresponding to the loss of birefringeance by 95% of the starch granules.

Starch gelatinisation characteristics can also be studied by using differential scanning calorimetry (DSC). During this process, an enthalpy change is measured. Samples are heated at a controlled rate (e.g. 10°C min^{-1}) in an oven and when starch starts to gelatinise, energy is taken up from the oven, without a concomitant temperature rise. The temperature of the reference sample in the oven keeps increasing. From the temperature difference between the feed sample and the reference sample related in time, and the released power from the oven, the enthalpy used for the reaction can be calculated. Cone and Wolters (1991) reported gelatinisation enthalpy values and transition temperatures for several starch sources (Table 6). Isolated starch was obtained after subjection to a sedimentation procedure (Cone and Wolters, 1991). The values for isolated starch in Table 6 belong to the starch granule fraction which was sedimented within an hour.

Table 6. Gelatinisation characteristics for raw feedstuffs and their isolated starch fraction.

Starch source	Raw feedstuff		Isolated starch	
	δH (J/g starch)	Tt (°C)	δH (J/g starch)	Tt (°C)
Potato	20.8	62.3	21.2	63.3
Faba bean	15.9	69.8	20.7	67.6
Sorghum	24.5	77.3	20.1	73.8
Maize	11.5	69.8	19.6	65.7
Pea	14.9	67.9	-	64.9
Wheat	12.8	62.2	16.7	57.9
Barley	14.1	61.2	15.9	57.9
Oats	15.2	64.9	15.2	62.4
Tapioca	14.3	71.0	15.1	68.0
Rice	4.7	64.8	5.2	55.3

Source: Cone and Wolters (1991).

Rice shows a small enthalpy change, both in the whole feedstuff as well as in the isolated starch. Enthalpy changes showed a negative correlation with starch degradability with α-amylase (–0.71 and –0.82) and with rumen fluid (–0.39 and –0.59) for raw feedstuffs and isolated starch respectively. This means that a higher degree of starch gelatinisation results in a better starch degradation. The DSC curve shows the magnitude of the enthalpy change and the temperature at which this change occurs. An example is given in Figure 4.

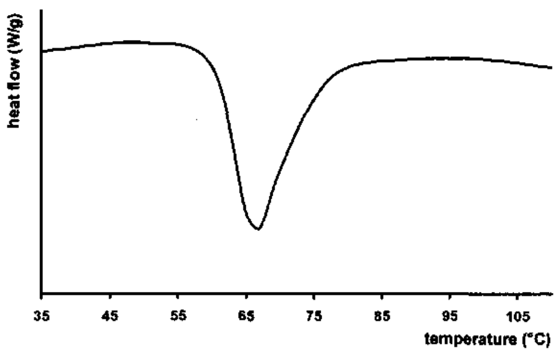

Figure 4. Example of a DSC curve.

Another way of studying starch properties is by measuring its rheological behaviour (viscosity changes) during a programmed heating-cooking-cooling cycle. The Brabender Viscoamylograph is an instrument that can be used for this purpose. This device measures the viscosity of starch-water dispersions that are stirred and heated at a uniform rate (1.5°C min^{-1}), held at any desired temperature (usually 90 or 95°C) for a specific time (20 to 60 min), and then cooled to 50 or 25°C at a uniform rate (1.5°C min^{-1}) and held at that temperature for a specific time (Swinkels, 1985). Brabender viscosity curves are characteristic for each type of starch. The viscosity starts to increase when considerable granule swelling takes place. The rise in viscosity can be faster or slower and the peak viscosity also differs among feedstuffs. Potato starch has a very high peak viscosity, tapioca and waxy starch sources show an intermediate peak while maize and sorghum starch have a low and wheat starch a very low viscosity peak. When the temperature increases further, the cohesive forces of the swollen granules weaken and the structure starts to collapse resulting in a drop in viscosity. The starch sources with the highest peak viscosities show the fastest decline in viscosity upon further heating. Upon cooling the viscosity rises again due to starch retrogradation (Swinkels, 1985).

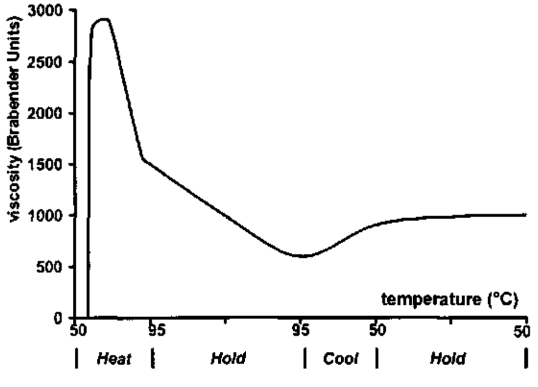

Figure 5. Example of a Brabender Viscosity Curve (taken from Swinkels, 1985).

Water Holding Capacity (WHC), Solubility, Swelling Power and Critical Concentration Values are other parameters that can be determined on the starch fraction (see Cone and Wolters, 1991 and Swinkels, 1985). Potato starch distinguishes itself from the other starch sources by showing an extremely high value for swelling power (weight of swollen granules per gram dry starch) and a very low value for the critical concentration (grams dry starch needed in 100 ml water to produce a paste in which the swollen granules occupy the whole volume at 95°C).

Many differences between feedstuffs do exist: they differ in cell wall composition, endosperm structure and composition and structure of the starch granules. These differences are likely to be responsible for the fact that different starch sources show different gelatinisation- and hydrolysis behaviour.

Starch digestion in poultry

The process of starch digestion

In poultry, no enzymatic hydrolysis of starch occurs prior to the stomach. Birds do not produce salivary α-amylase. After the ingested feed has passed the crop and proventriculus, it enters the gizzard. In the gizzard the feed is ground before it passes to the small intestine. Pancreatic α-amylase is secreted in the lumen of the small intestine. The greatest α-amylase activity is in the jejunum and it is generally believed that α-amylase is produced in excess of requirement (Rogel et al., 1987). The optimal pH for α-amylase is 6.9. When α-amylase reaches the starch fraction then starch hydrolysis can begin. During starch digestion by α-amylase, amylose is broken down to maltose and maltotriose. Amylopectin is degraded to maltose, maltotriose and α-dextrins. An extensive description of the digestion of amylopectin is given by Moran (1982). Alpha-amylase attaches to the substrate at a random position along the α-1,4 chain (Figure 6). The attachment takes place through linkages between the catalytic sites of the enzyme and five glucose units. This attachment is followed by a cleavage between the second and third α-1,4 linked glucose unit. The enzyme stays on the fragment towards the non-reducing end and 'slides over' the chain in order to refill its sub-sites and starts with another cleavage, releasing maltose. At the end of the chain a maltotriose molecule remains. α-Amylases possess less specificity for smaller glycosidic oligosaccharides. In the case of these small molecules only two or three catalytic sites are occupied and therefore it is impossible to span a cleaving site. In addition, α-amylase does not possess the specificity for the α-1,6 bonds at the branching points in amylopectin and the ability to break the α-1,4 linkages adjacent to the branching point is prevented by its spherical structure (Gray, 1992). Among the endproducts of amylopectin digestion by α-amylase are α-dextrins that contain α-1,6 linkages. The smallest molecule is a tetrasaccharide (Moran, 1982). The endproducts of α-amylase digestion cannot pass the intestinal wall. These molecules must therefore be further degraded to glucose molecules. Alpha-amylase is the only carbohydrase which is dissolved in the fluid in the lumen of the small intestine. Maltose, maltotriose and α-dextrins are hydrolysed by oligosaccharidases (glycoproteins) which are located in the intestinal surface brush border membrane. Glucoamylase is an enzyme that removes single glucose units from the non-reducing end of the α-1,4 chain. This enzyme has the same limitations as α-amylase. Sucrase-α-dextrinase is a hybrid carbohydrase that is synthesised as a single glycoprotein chain. This enzyme is cleaved in sucrase and α-dextrinase units by pancreatic proteases.

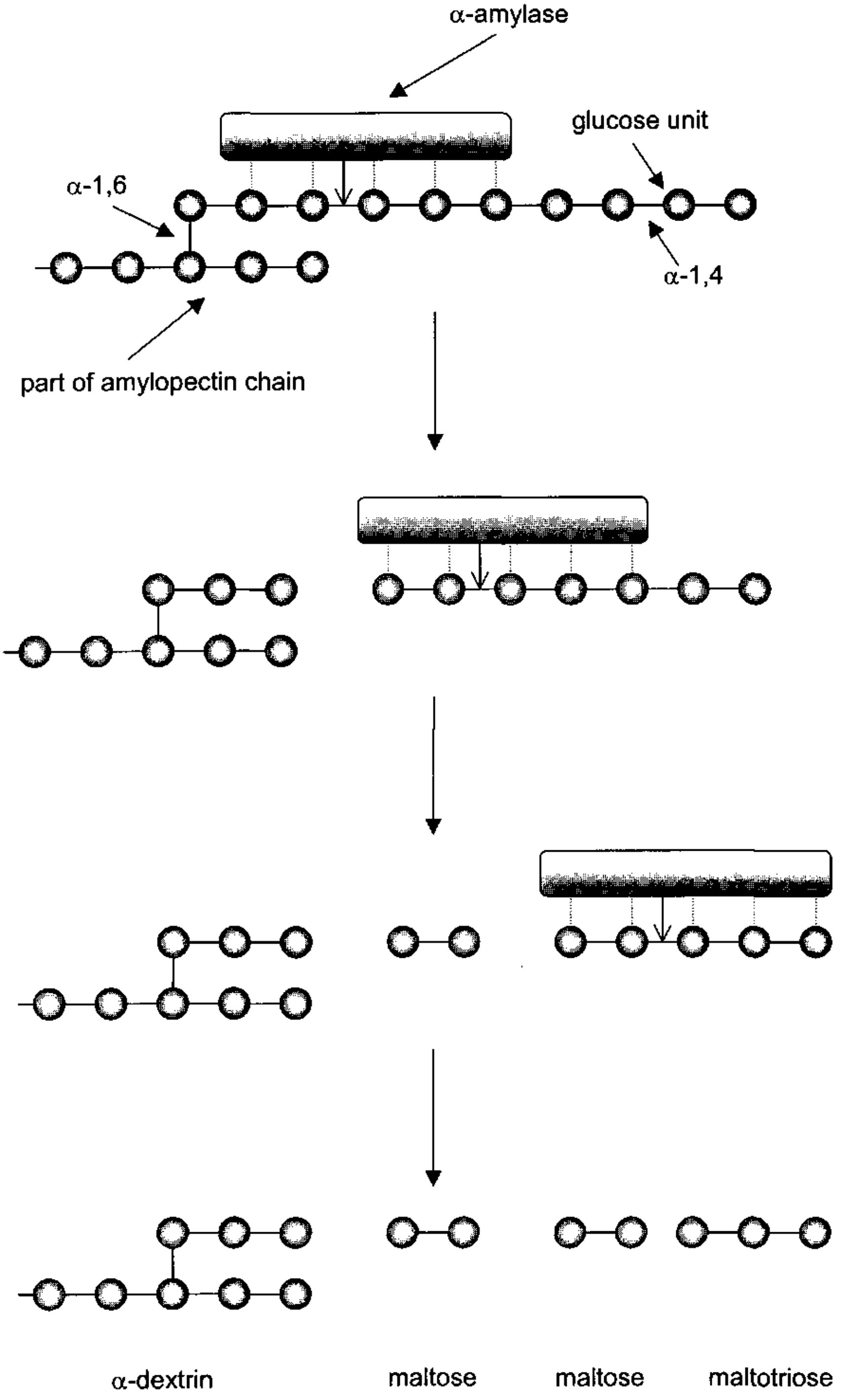

Figure 6. Schematic presentation of the initial enzymatic hydrolysis of amylopectin by α-amylase.

Sucrase is an efficient α-1,4 glucosidase which complements the specificity of glucoamylase by hydrolysing the shorter α-1,4 bound oligosaccharides (especially maltose and maltotriose because of a preference for shorter chains) into glucose units. Alpha-dextrinase (isomaltase) is capable of cleaving the non-reducing end α-1,6 bond, leaving maltose, maltotriose and glucose. The glucose molecules resulting from these enzyme actions have to be absorbed through the intestinal wall so that they can be utilised in the intermediary metabolism. The brush border of the small intestine contains a glycoprotein with a high affinity for glucose. This specific glucose carrier transports the glucose molecules through the gut wall. The carrier depends on the presence of Na^+ in the glucose solution of the lumen. Two Na^+-ions bind to one side of the carrier apart from glucose and are carried and released inside the cell together with a glucose molecule. The driving force for this transport is the Na-K-pump which pumps the Na^+ back again. At the basolateral surface the glucose is likely to diffuse to the capillaries, but it is also possible that an interaction with a second carrier protein is involved at the basolateral surface (Gray, 1992). Part of the absorbed glucose will serve as an energy source for the gut wall and will therefore be oxidised. The remainder is taken up by the bloodstream (via the portal vein) and supplies energy for other tissues or is built in glycogen or fat for future energy demands.

Resistant starch

Part of the ingested starch passes through the small intestine without being degraded by α-amylase. This fraction is referred to as resistant starch (RS), a term that was introduced by Englyst in 1982. RS can be fermented to volatile fatty acids (VFA) and other fermentation products by the micro-flora present in the caeca and colon. The size of the RS fraction depends on both feed properties and animal factors. Feed properties include the physical and chemical characteristics of the feedstuff and the starch granules in the feedstuff. Feed properties can change during milling- and heat treatments. Animal factors are: enzyme activity,absorption capacity and the feed transit time in the gut. These animal factors are affected by diet and feed intake pattern. A distinction should be made between analytical resistant starch, which is the starch fraction that resists long term enzymatic attack *in vitro*, and physiological resistant starch, which is the starch fraction that escapes enzymatic digestion in the small intestine of the animal. Englyst et al. (1992) have defined three RS fractions:

RS_1: Physically inaccessible starch. Plant cell walls and protein structures protect this starch fraction from enzymatic attack. This fraction is bigger in coarsely ground feedstuffs.

RS_2: Resistant starch granules. These are found in unheated feedstuffs or feedstuffs heated under low moisture conditions.

RS_3: Retrograded starch. This retrograded starch is mainly retrograded amylose and can be found in feedstuffs that were heated under high moisture conditions and high temperatures. Higher RS_3 fractions can be expected in feeds that have been subjected to more than one heat treatment.

These three fractions can be determined in the laboratory and together they form the total RS fraction. Retrograded starch is probably the smaller portion of resistant starch in feeds. Physically inaccessible starch can increase the total amount of resistant starch considerably (Annison and Topping, 1994). An example of resistant starch granules are the ones in raw potatoes and unripe bananas. Fermentation of RS in the hind gut is stimulated by the presence of complex carbohydrates like starch itself and NSP and is also dependent on the passage rate of the digesta. The main VFA produced during fermentation are acetic acid, propionic acid and butyric acid. These three VFA make up more than 90% of the fermentation products. The other 10% is formed by carbondioxide, hydrogen and methane. The VFA are absorbed from the hind gut, enter the portal circulation and are transported to the liver. The VFA have a beneficial effect on the health of the large bowel by inhibiting pathogen growth, increasing fluid- and electrolyte absorption and reducing the intestinal pH. It is likely that starch from different plant origin differ considerably in their fermentative characteristics (Annison and Topping, 1994).

Measurement of starch digestion in poultry

A distinction should be made between total starch digestion and ileal starch digestion. Indigestible indicators (chromic oxide, titanium oxide, HCl-insoluble ash, etc.) are often used in digestion trials with poultry. Total starch digestion is determined by analysing the content of starch and the indicator in both the diet and the excreta. It is assumed that the indicator and the nutrients pass the intestinal tract with the same rate. Total starch digestion can be calculated from the change in the starch to indicator ratio. Total starch digestion indicates how much starch is absorbed from the total gastrointestinal tract. This figure gives no information about the amount of starch that is absorbed as glucose from the small intestine or the amount that is fermented to volatile fatty acids that are absorbed in the hind gut. For the determination of ileal starch digestion more sophisticated techniques are required. In digestion trials with poultry the slaughter technique is often used for this purpose. The animals are euthanised at the end of the experimental period, (part of) the small intestine is removed from the animal and the contents are collected for analysis. This method also requires the use of an indigestible marker. The use of the slaughter technique offers the benefit that digesta can be collected from any part of the gastrointestinal tract. Digestion of starch (and other nutrients) can be monitored along the whole gastrointestinal tract. Two diets can contain starch that is completely digested, but be different in the amount of starch that is digested in a specific segment of the small intestine. Differences in the amount of starch digested in specific segments of the small intestine indicate that the susceptibility of the starch fraction to enzymatic attack differs. This implies differences in starch digestion rate. Starch digestion rate can be measured when the starch digestion in the successive segments are related to the retention time of the digesta in these segments. It should be realised though, that digestion coefficients obtained by the slaughter technique are average values for the segment from which the digesta was collected. Therefore, for ileal starch digestion the segment at the end of the ileum from which digesta is collected should not be too long. Otherwise, the ileal starch digestion coefficient can be underestimated. Care should be taken when the digesta is collected, since only one sample can be taken per animal and that sample should be as representative as possible. Animal effects can be minimised by pooling digesta from several birds in the same experimental unit. When digestibility coefficients are presented, it is important to give information about the technique that was used, animal weight (or age) and feed processing. Feed processing is not always given, which makes it difficult to compare results from different experiments. For instance, it is not always clear how feedstuffs are ground (mill type, screen size) and whether or not feeds were pelleted.

Starch digestion of different feedstuffs in poultry

Rogel et al. (1987) measured starch content and faecal starch digestibility in 38 wheat batches grown in Australia in 1984. Wheat from these batches were included in diets which were pelleted without steam addition. Starch content of the wheat samples varied from 504 to 596 g/kg (air dried) and total starch digestion in 6 week old broilers ranged from 82 to ± 100%. The starch fractions from two poorly digestible wheat batches were isolated and the isolated starch was incorporated in diets at levels of 55%. This time, digestion was almost complete for starch from both wheat batches. These results suggest that the starch fraction in wheat with a low energetic value is less accessible for the digestive enzymes in the gut.

In Tables 7 and 8, starch digestion of several starch sources is presented. In some experiments digestion of isolated starch fractions is measured, in other experiments digestion of starch in its original matrix is measured. Differences in digestion of isolated starch fractions can be explained by starch composition and -structure. Differences in digestion of starch in its original matrix are related to both differences in the matrix and the starch itself. The digestion coefficients clearly show that starch digestion of some feedstuffs are affected by processing and age of the bird. Starch digestion is determined by the interplay of starch degrading enzymes and the starch itself. Enzyme activity in the small intestine is increasing in the young bird (Nitsan et al., 1991). Accessibility of starch for enzyme attachment is affected by physical and chemical structures in the feedstuff and processing.

Yutste et al. (1991) studied the digestibility of isolated starch from maize, wheat, cassava, pea, faba bean and potato in young chicks and adult cockerels. Differences found in these experiments are mainly associated to starch structure characteristics. NSP- and protein fractions associated to the starch granules in the feedstuff could not affect starch digestion in this experiment. Starch from different sources (feedstuffs) has different structures and amylose to amylopectin ratios. Differences in granule size have also been observed. The observed differences in this trial are in line with differences in starch characteristics described in literature. Maize-, wheat- and cassava starch express the A-type starch, which is easier to digest than B and C- starch.

Table 7. Starch digestibility of various feedstuffs in poultry found in literature.

Feedstuff	Treatment	Age	Starch digestion Ileum	Excreta	Source
Wheat	Isolation	2 wk	97.6	96.3	1
	Isolation	3 wk		99.4	1
	Isolation	Adult		99.0	1
	Ground: 3mm	Adult		96.4	2
	Pelleted and ground: 2mm	Adult		97.5	2
	Pelleted and ground: 2mm	Adult		97.2	2
	Crushed	3 wk		77.2	3
	Crushed + cold pelleted	3 wk		97.8	3
	Crushed	7 wk		95.1	3
	Crushed + cold pelleted	7 wk		94.4	3
Maize	Isolation	2 wk	97.8	96.0	1
	Ground: 2mm	Adult		98.1	2
	Pelleted and ground: 2mm	Adult		98.2	2
	Double pelleted and ground: 2mm	Adult		98.3	2
	unknown	3 wk		97.9	4
Barley	Ground: 3.5mm low viscosity	19 d	88.5	98.5	5
	Ground: 3.5mm high viscosity	19 d	85.1	98.6	5
Cassava	Isolation	2 wk	94.7	95.0	1
	Isolation	3 wk		99.3	1
	Isolation	Adult		99.2	1
Pea	Isolation	2 wk	94.4	93.2	1
	Isolation	3 wk		94.2	1
	Isolation	Adult		98.0	1
	Ground: 0.5mm	4 wk	93.8	96.8	6
	Whole	Adult		75.6	7
	Ground: 1mm	Adult		88.1	7
	Heated: 121°C/30 min and ground	Adult		90.4	7
	Heated: 121°C/30 min/1 bar and ground	Adult		91.2	7
	Dehulled + ground	Adult		92.8	7
	Ground: 2mm	Adult		91.5	2
	Pelleted and ground: 2mm	Adult		95.9	2
	Double pelleted and ground: 2mm	Adult		97.1	2
Faba bean	Isolation	2 wk	72.3	69.2	1
	Isolation	3 wk		78.2	1
	Isolation	Adult		94.5	1
	Tannin free	3 wk		79.6	4
	Tannin free, heated: 120°C/30 min	3 wk		90.3	4
	Tannin containing	3 wk		92.9	4
	Tannin containing, heated: 120°/30 min	3 wk		95.7	4
Potato	Isolation	2 wk	43.9	36.8	1
	Isolation	3 wk		39.3	1
	Isolation	Adult		70.2	1

Ileal digestibility is measured by the slaughter technique. 1) Yutste et al., 1991, 2) Carré et al., 1987, 3) Rogel et al., 1987, 4) Guillaume, 1978, 5) Hesselman and Åman, 1986, 6) Treviño et al., 1990, 7) Longstaff and McNab, 1987.

Potato starch is of the B-type, which is much more stable and finally pea- and bean starch show the C-type which has a susceptibility to enzyme attack in between that of A and B. Furthermore, amylose content differs between cereal starch and legume starch. Starch with high amylose contents are less easily digested. The highest amylose content is found in legumes (±33%) and cereals show an average amylose content of ± 25%. Cassava and potato starch contain about 18% amylose. The cereals and tapioca have relatively small granules and the starch granules in feedstuffs like faba beans, peas and potato (less digestible in this trial) have bigger granules. Franco et al. (1992) observed that small starch granules were hydrolysed rapidly compared to big ones. From these observations it can be concluded that characteristics of the starch granules itself can explain (part of) the differences found in starch digestibility of different feedstuffs.

However, other properties of the feedstuff should also be considered. The first step in the hydrolysis process is the attachment of enzymes to the substrate. Attachment can only take place when the substrate is accessible for enzymes. Accessibility depends on other components of the feedstuff (like cell wall components) and whether or not the feedstuff has been ground. The experiment of Longstaff and McNab (1987) on pea starch digestibility clearly shows the importance of grinding (Table 7). When the whole feedstuff was fed, starch digestibility was low (76%). When the peas were ground to pass a 1 mm screen, starch digestibility increased dramatically (to 88%). Heat treatment prior to grinding in order to gelatinise the starch fraction did not increase starch digestibility much further. When the peas were dehulled prior to grinding, starch digestibility increased to 93%. Apparently, cell walls in the hulls were responsible for a limited accessibility of the starch fraction in peas. In this experiment the problem of accessibility was clearly demonstrated. The heat treatments of peas in this experiment did not improve starch digestibility very much. Carré et al. (1987) treated feeds with wheat, maize and peas as the starch sources in two ways, steam pelleting and double steam pelleting (with a grinding step in between). These heat treatments did not affect starch digestibility in the maize and wheat diets. Starch digestibility in diets with peas and wheat or peas and maize improved significantly after these heat treatments. When pea starch digestibility was calculated back from total starch digestibility in the diet and starch digestibility found in the maize and wheat diets, pea starch digestibility increased on average from 92 to 96 and 97% for pelleting and double pelleting respectively. This is probably a combined effect of starch gelatinisation due to steam pelleting and the shear forces during the grinding steps and pelleting. Effect of autoclaving (120°C for 30 min) on starch digestibility of faba beans in 3 week old broilers was studied by Guillaume et al. (1978). Maize starch digestibility served as a reference and was 98%. Tannin free and tannin containing field beans were compared. Starch digestibility of faba beans was 80% for raw tannin free beans and 93% for tannin containing beans (when beans were the only starch source in the diet). Autoclaving at 120°C for 30 min resulted in a starch digestibility of 90% for tannin free and 96% for tannin containing beans.

In this experiment starch digestibility was improved by heat treatment. The observation that the tannin free line showed a lower starch digestibility was surprising. The author mentions lectins as a possible explanation for this. Accessibility is not only affected by direct feed properties like cell wall structures and particle size but can also be affected indirectly. Wheat, rye and triticale may contain considerable amounts of arabinoxylans and the cell walls in the endosperm of barley and oats contain considerable amounts of β-glucans. These components cannot be digested by the enzymes produced by the bird. The insoluble part of these non starch polysaccharides (NSP) can act as a physical barrier to enzymes. A fraction of the arabinoxylans and β-glucans solubilise after ingestion. Diets with considerable amounts of soluble NSP may increase viscosity of the digesta in the intestines. A high digesta viscosity may reduce the diffusion rate of enzymes and increase the feed passage time (Classen, 1996). As a result, starch accessibility is impaired in this situation. Hesselman and Åman (1986) studied starch digestibility for barley with high and low viscosity with or without β-glucanase in 19 day old broilers of mixed sex. Barley starch was degraded slowly, but completely when β-glucanase was included in the feed (not presented in table 7). When the enzyme was not included, the barley starch was not completely digested in the small intestine. Without enzyme supplementation, starch digestion rate was higher for the low viscosity barley. The high viscosity barley was harvested in the early yellow stage, while the low viscosity barley was harvested at the combine stage and is therefore more representative for practical situations. The low viscosity barley showed a slightly higher β-glucan content than the high viscosity barley (5.2 vs. 4.5 % on DM basis). No information regarding the soluble fraction of the β-glucans is given.

Table 8 shows the results of experiments in which starch digestion was measured in different segments of the small intestine. Site of starch digestion can be an indication for starch digestion rate. However, it should be realised that differences in passage rate can affect site of starch digestion. The digestibility studies cited here did not include measurements on passage rate, therefore reliable statements about rate of digestion cannot be made. The chosen segments were not the same in the different experiments, therefore sound comparisons cannot be made. The results presented in Table 8 indicate that digestion of isolated maize- and wheat starch is relatively rapid. The digestible starch fraction was completely digested prior to the distal ileum. Cassava- and pea starch digestion was slightly slower and less complete. Starch digestion in faba bean- and potato starch was considerably slower than in the other isolated starch samples. The extremely high standard deviation for potato starch may explain the decreasing values for starch digestion along the digestive tract. In the experiment by Treviño et al. (1990), the peas were finely ground (to pass a 0.5 mm screen). Starch digestion prior to the ileum was 75% and this increased to 94% at the end of the ileum. Unfortunately the gut segments differed too much to make comparisons with the isolated starch in the experiment by Yutste et al. (1991). The experiment of Hesselman and Åman (1986) clearly shows that starch in low viscosity barley is digested faster than starch in high viscosity barley. The trial in which the rye / wheat based diet was studied was set up the same way as the barley trial. From these figures it appears that barley has a slower starch digestion rate than wheat and rye. However, as Rogel et al. (1987) already found, large differences between grain batches can be expected.

Table 8. Site of starch digestion (%) in different starch sources.

Feedstuff	Segment of small intestine		
	1	2	3
Maize starch[1]		96.2	97.8
Wheat starch[1]		97.3	97.6
Cassava starch[1]		90.5	94.7
Pea starch[1]		90.3	94.4
Bean starch[1]		59.9	72.3
Potato starch[1]		58.1	43.9
Pea[2]		75.3	93.8
Barley (low viscosity)[3]	35.4	62.6	88.5
Barley (high viscosity)[3]	4.4	44.2	85.1
Rye / wheat (50/50)[4]		85.6	96.5

[1] Age: 2 weeks; segment 2: anterior part of ileum; segment 3: posterior part of ileum (Yutste et al., 1991).
[2] Age: 4 weeks; segment 2: jejunum; segment 3: ileum (Treviño et al., 1990).
[3] Age: 19 days; segment 1: first part of small intestine (SI); segment 2: second part of SI; segment 3: last part of SI (Hesselman and Åman, 1986).
[4] Age: 20 days; segment 2: second part of SI; segment 3: last part of SI (Petterson and Åman, 1989).

Adult cockerels are able to digest starch from wheat, cassava and peas very well (98-99%). Digestion of starch from faba bean is slightly lower (95%) and potato starch is hard to digest for these animals (70%). Broilers never reach the adult age. The results from Yutste et al. (1991) indicate that even young chicks are capable of digesting maize-, wheat- and cassava starch (and, to a lesser extent, pea starch). However, these animals have more problems digesting starch from faba bean- and potato starch (significant age effect). The fact that faba beans and potatoes are less well digested by young chicks than adult cockerels suggests that the development or adaptation of the digestive system of young chicks is not completed.

The most likely cause for incomplete pea starch digestion in chickens is the accessibility for the enzymes. Rogel et al. (1987) showed that starch from wheat in unpelleted diets was poorly digested (77%). Cold pelleting improved starch digestibility (98%) and reduced the variation between birds. No differences in starch digestion were found between pelleted and unpelleted diets in 7 week old broilers. Cold pelleting did not gelatinise wheat starch. The improvement in starch digestibility in the young chicks can probably be attributed to particle size reduction caused by shear forces that are involved with pelleting.

A digestive system that is not completely developed or adapted to starch rich diets appears to be capable of digesting readily accessible and digestible starch structures. However, digestion of more resistant and less accessible starch structures appear to be problematic in these circumstances.

When starch digestion in the small intestine is incomplete, starch fermentation in the hind gut may occur. However, in the case of the isolated starch samples, no fermentation in the caeca or colon took place. Treviño et al. (1990) observed a higher starch digestibility in the caeca (97%) than in the ileum (94%). However, the sample was taken from digesta collected from the complete ileum (from diverticulum to the end of the small intestine). The third segment of the small intestine in the experiment of Treviño et al. (1990) corresponds to segments 2 and 3 combined in the experiment of Yutste et al (1991). The digestibility value obtained is an average value for the gut segment and starch digestibility at the terminal ileum may have been as high as in the caeca. Hesselman and Åman (1986) observed for barley that starch digestibility in the last part of the ileum was incomplete and that starch digestion in colon samples was complete. A possible explanation is that in the case of barley, the starch granules were rather inaccessible due to the surrounding cell walls which prevented starch digestion in the small intestine. These cell wall components might have been broken down by the microbes in the lower tract, thereby exposing the well digestible starch granules to the digestion process.

Conclusions

Based on starch digestibility figures reported in literature, it appears that different starch containing feedstuffs are degraded at different rates and to a varying extent in poultry. The most important parameter is ileal starch digestion. The energetic value of starch is highest when starch is digested and absorbed in the small intestine. In contrast to pigs, the resistant starch fraction is not fermented in the hind gut of broiler chickens. In poultry, ileal starch digestion should be maximised. Starch digestion rate may be important when the feed residence time in the small intestine is limiting starch digestion. In broilers, the mean retention time in the small intestine is approximately 3 hours, which is relatively short.

The extent of starch digestion in an animal depends on animal factors and dietary factors. Examples of animal factors are enzyme activity, absorption capacity and feed transit time. However, feed transit time depends also on diet composition. Diet composition also affects digesta properties. The differences in rate and extent of starch digestion between feedstuffs are related to certain characteristics of the starch source. In order to be able to digest the starch fraction in a feedstuff, the enzymes should have proper access to the starch fraction. Accessibility is a key factor for digestive behaviour in the alimentary tract (Figure 7).

Accessibility is determined by particle size and composition of the starch source. When accessibility is limited by other feed components like cell walls or protein fractions, then starch digestion will be slower. Thus, composition of the feedstuff is one important factor.

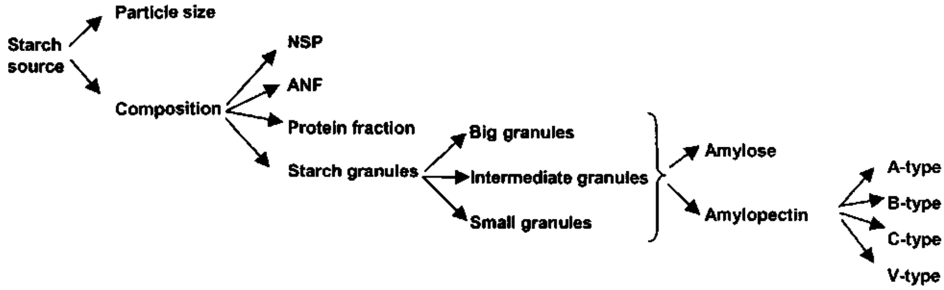

Figure 7. Factors affecting rate and extent of starch digestion in the alimentary tract of poultry.

When the effect of other materials than starch is eliminated there are still differences in starch digestibility between different starch sources. This is caused by differences in the starch granule itself. Granule size affects starch degradation rate, but also granule composition: amylose to amylopectin ratio. Finally, the structure of amylopectin has a major effect on starch digestibility, since the crystalline structure of starch is caused by the amylopectin fraction. Three different structures have been found in amylopectin: A, B and C type amylopectin.

Rate and extent of starch digestion can be altered by means of feed processing. A distinction can be made between particle size reduction and moist-heat treatment. When starch containing feedstuffs are subjected to a treatment which results in particle size reduction (grinding, pelleting, etc.), then protective layers are destroyed, relative surface area is increased and starch granules may be damaged. These effects facilitate starch digestion. Heat treatments in combination with sufficient moisture results in starch gelatinisation. During this process the crystallinity of amylopectin disappears.

It can be concluded that starch digestibility is high for cereal grains and cassava. Longstaff and McNab (1986) state that starch gelatinisation is not necessary for an effective starch digestion in poultry diets. This statement holds for cereal grains and cassava. However, the results of Carré et al. (1987) and Guillaume (1978) show that heat treatment of feedstuffs with a low starch digestion (pea, faba bean) improves starch digestion considerably.

Chapter 3

Starch Digestion Rate in the Small Intestine of Broiler Chickens Differs among Feedstuffs

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Abstract

Dietary starch is the major energy source for broiler chickens, and knowledge about its digestive behavior can be important. In a digestibility trial with 720 broiler chickens, site, rate and extent of starch digestion were measured for 12 feedstuffs. Starch digestion was determined using the slaughter technique, which involves removal of the small intestine from the recently killed chicken, with manual collection of the contents. Starch digestion coefficients were calculated from remaining starch in three segments of the small intestine and in excreta. Mean retention time in four segments of the small intestine was measured. This enabled calculations for starch digestion rate (k_d). Ileal starch digestibility varied from 33% (potato starch) to 99% (tapioca). Retention time for digesta in the post duodenal small intestine varied from 136 min (barley diet) to 182 min (potato diet). On the basis of starch digestion rates, a distinction was made between slowly digestible starch (k_d < 1 /h), gradually digestible starch (k_d: 1-2 /h) and rapidly digestible starch (k_d > 2 /h). Starch from common beans was digested most slowly (k_d: 0.5 /h), and starch from tapioca was digested most rapidly (k_d: 4.3 /h). Starch digestion rate of potato starch and legume seeds was lower than that of cereal grains and tapioca. Degradation of starch entering the hind gut of the birds did not occur. Milling (roller milling compared to hammer milling) of maize affected rate, but not the extent of starch digestion. We concluded that site of starch digestion within the small intestine is not an accurate indicator for starch digestion rate.

Keywords: starch, broiler chickens, digestion rate, retention time

Introduction

The apparent metabolizable energy (AME) content of broiler feeds is determined by measuring the energy value of the feed minus fecal- and urinary energy (Fisher, 2000). The feed industry uses regression equations based on digestible nutrients to predict the AME content of feedstuffs. Studies have shown that the AME of wheat is positively correlated with starch digestibility (Rogel et al., 1987, Wiseman et al., 2000) and inversely correlated with nonstarch polysaccharides (NSP) content (Choct et al., 1999). In The Netherlands, crude protein, crude fat and nitrogen-free extract (NFE) are used in the AME formula (Centraal Veevoederbureau, 1999). Starch, the major energy supplier for broiler chickens, is not included as such, but it is part of the NFE fraction. This has consequences for the nutritional value, especially when the starch proportion in the NFE fraction is variable.

Incomplete starch digestion in broiler chickens was observed for wheat (Rogel et al., 1987), barley (Hesselman and Åman, 1986), peas (Longstaff and McNab, 1987) and isolated starch from several feedstuffs (Yutste et al., 1991). According to Moran

(1982), starch digestibility is a function of granule surface area, starch structure and degree of crystallinity. Granule size differs among feedstuffs, and small granules are generally digested more rapidly than larger granules (Franco et al., 1992). Starch granules are composed of amylose and amylopectin molecules. Starch with a high amylose content is often considered to be less digestible. Starch structure is divided in A-, B and C-type starch. A-type starch is more susceptible to enzymatic attack than B-type starch, and C-type starch is intermediate (Oates, 1997). Factors not directly related to starch itself may also affect its digestibility. Starch granules can be encapsulated by a rigid protein matrix or by cell walls from the same feedstuff. This reduces the accessibility of starch granules to enzymatic attack (Classen, 1996). Furthermore, other ingredients of the diet may also affect starch digestion. Soluble NSP in the diet increase digesta viscosity, possibly impairing starch digestion (Classen, 1996, Refstie et al., 1999). Starch digestion is also affected by animal-related factors, including age, feed intake, passage rate and absorption capacity. In human nutrition, much work has been done in the area of starch digestion. In this field, the term resistant starch (RS) was introduced. EURESTA (acronym for European Resistant Starch research group) defined RS as "... the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Asp, 1992). Englyst et al. (1992) partitioned RS into three separate fractions, i.e., physically inaccessible starch (RS_1), resistant starch granules (RS_2) and retrograded amylose (RS_3). Differences in the extent of starch digestion might be explained by the existence of an enzyme-resistant starch fraction, differences in starch digestion rate or both. There are indications that starch digestion rates differ among feedstuffs (Yutste et al., 1991). The dynamics of starch digestion may have considerable nutritional consequences. Rapid- or slow starch digestion may elicit different metabolic responses in the animal (e.g., synchronization of protein and starch digestion, effect on insulin response, microbial fermentation). The objective of this experiment was to study the site, rate and extent of starch digestion of 12 different feedstuffs in broiler chickens. These *in vivo* starch digestion data will also serve as reference values for the development of an *in vitro* method that simulates passage through the alimentary tract of broiler chickens. Therefore, feedstuffs were selected to cover a wide range in starch characteristics such as starch structure, amylose content and granule size.

Materials and methods

Experimental design

In an *in vivo* experiment, starch digestion of 12 feedstuffs was studied. The experiment was performed with two batches of 360 female Ross broiler chickens (obtained from Cobroed, Lievelde, The Netherlands), housed in 36 pens. An experimental unit was formed by one pen containing 10 birds. The experiment was conducted in two periods (one batch per period). Each experimental treatment consisted of six replicates equally assigned to the periods.

Animals, housing and diets

The experimental protocol was in agreement with the standards for animal experiments and was approved by the Ethical Committee of De Schothorst. Newborn chicks were kept in a warm environment (temperature decreased gradually from 30 to 24°C) and received a standard starter diet (supplied by feed cooperative Arkervaart-Twente, Nijkerk, The Netherlands) until they were 14 days old. At this age, chicks were assigned to dietary treatments on the basis of live weight and were transferred to battery cages (treatments equally distributed across floors). The cages were located in a room with 23 h light/d, and ambient temperature (± 22°C). The chicks were fed one of 12 experimental diets (supplied by feed cooperative Arkervaart-Twente), varying in starch source until the end of the experiment. Birds were given free access to the diets, which were provided as a mash. Composition of the starch sources examined is presented in Table 1; the composition of the experimental diets is presented in Table 2.

Table 1. Composition of starch sources (as analyzed).

Starch source	Dry matter	Ash	Crude protein	Crude fat	Crude fiber	Starch	Free glucose
				g/kg product			
Wheat	873	16	115	15	29	539	8
Maize, hammer-milled	872	14	81	40	26	610	8
Maize, roller-milled	869	16	86	41	26	609	8
Maize, waxy	854	15	88	50	27	580	8
Common beans, heat-treated	843	54	215	19	40	311	10
Barley	853	22	103	18	46	488	7
Sorghum	856	14	78	30	25	616	4
Peas	871	39	215	12	56	373	11
Horse beans	807	32	209	9	80	347	9
Tapioca pellets	886	61	23	4	48	617	3
Raw potato starch	800		1		3	774	0
Brown rice, not polished	861	13	85	21	13	701	4

All dietary starch originated from these 12 feedstuffs. Feedstuffs were incorporated into the diets to obtain similar starch contents. As can be seen from Table 2, most diets contained only one starch source. Four diets contained two starch sources. Peas, common beans, horse beans and potato starch were each incorporated to a limited extent into the diets (250-300 g/kg) to avoid digestive disorders. Each of these diets was supplemented with common maize (hammer milled) to achieve a starch content similar to the other diets. Three different maize diets were fed, differing in variety and milling treatment (Table 1). All starch sources were milled by a hammer mill to pass a 2.75-mm screen. In addition, as a separate treatment, common maize was also roller-milled. Diets contained Cr_2O_3 as an indigestible marker (Table 2).

Table 2. Composition of experimental diets.

Feedstuff / nutrient	A	B	C	D	E	F	G	H	I	J	K	L
	Treatment g/kg product											
Wheat	551.5											
Maize, hammer-milled		519.5			352.5			313.5	348.0		188.0	
Maize, roller-milled			519.5									
Maize, waxy				519.5								
Common beans, heat-treated					300.0							
Barley						633.5						
Sorghum							521.0					
Peas								300.0				
Horsebeans, white									300.0			
Tapioca, dehydrated										492.0		
Potato starch, dehydrated											250.0	
Brown rice, not polished												519.9
Soybeans, extracted	221.0	285.5	285.5	285.5	152.0	162.0	246.0	165.0	152.0	277.0	298.5	274.5
Soybean hulls	41.5	30.0	30.0	30.0	14.0		49.0	36.5	14.0	17.5	51.0	58.5
Meat meal	60.0	60.0	60.0	60.0	60.0	70.3	60.0	60.0	60.0	48.0	72.0	60.0
Feathermeal, hydrolyzed	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Potato protein										29.4		
Soybean oil	25.4	14.5	14.5	14.5	17.0	21.8	20.0	18.1	16.5	31.0	20.6	41.0
Animal fat	53.5	45.0	45.0	45.0	58.1	61.0	53.9	61.2	64.0	61.7	76.1	
L-Lysine (25%)	3.3	0.2	0.2	0.2		8.3	4.8					
DL-Methionine (10%)					1.0							
Vitamin-mineral premix[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Monocalcium phosphate	3.6	5.7	5.7	5.7	5.6	2.5	5.9	6.0	5.4	6.5	5.8	7.4
Anticoccidial premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Ground limestone	3.5	2.9	2.9	2.9	3.1	4.1	2.7	3.0	3.5		1.5	1.9
Sodium chloride	0.2	0.2	0.2	0.2	0.2		0.2	0.2	0.1	0.4		0.3
Cr_2O_3	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Dry matter	890	886	885	877	878	878	881	838	872	898	879	883
Ash	54	56	58	57	61	55	56	56	52	73	56	56
Crude protein	223	234	244	237	220	206	220	230	216	218	225	232
Crude fat	104	98	99	102	108	107	100	108	110	106	122	69
Crude fiber	40	37	41	33	40	36	36	42	43	43	34	38
Starch	319	332	305	311	313	321	316	312	329	332	324	371
Free glucose	10	11	12	12	10	8	9	10	9	7	9	9

[1] The premix contained the following microelements (mg/kg) : Mn, 6000; Zn, 6000; Fe, 4000; Cu, 1000; I, 80; Se, 10; retinol, 300 ; cholecalciferol, 5 ; α-tocopherol, 2500; menadione, 130; riboflavin, 400; pyridoxine, 100; cyanocobalamin, 1.5; nicotinic acid, 3000; folic acid, 50; D-pantothenic acid, 700; choline, 22000; biotin, 4; Zn, Bacitracin, 5000.

[2] The premix contained (mg/kg): Lasalocid-Na, 18000.

Sample collection

Excreta were collected on d 27 and 28 for the determination of total starch digestion. This was done twice a day (every 4 h) to minimize changes in excreta composition after excretion. After collection, excreta were stored in the refrigerator (4°C) until d 29, when they were freeze-dried. On d 29, the birds were killed by an intravenous injection of T61, which is an aqueous solution containing 200 g embutramide, 50 g mebezoniumiodide and 5 g tetracainehydrochloride per liter (Hoechst Veterinär GmbH, München, Germany). Immediately after injection, the small intestine was removed. The mesentery was cut, and jejunum and ileum were separated at Meckel's diverticulum. Both jejunum and ileum were split into two parts of equal length, namely, anterior jejunum (AJ), posterior jejunum (PJ), anterior ileum (AI) and posterior ileum (PI). Digesta were rinsed out of each segment (without squeezing) with demineralized water (4°C) into separate aluminium containers. Digesta were stored at –20°C and subsequently freeze-dried. After freeze-drying the samples were pre-ground with a pestle and mortar and subsequently ground in a Retsch mill to pass a 1-mm screen. Samples were analyzed for starch (except the contents of the anterior jejunum) and Cr_2O_3.

Chemical analyses and measurements

Experimental diets and starch sources were analyzed for contents of dry matter, ash, nitrogen (Dumas), crude fat, crude fiber, starch and free glucose. Starch, free glucose and Cr_2O_3 were determined in experimental diets, and freeze-dried digesta and -excreta. Starch was analyzed according to Englyst et al. (1992). Cr_2O_3 content was determined by wet destruction with a mixture of $HNO_3/HClO_4$ (1:1). The absorption of the hexavalent Cr atom, measured at a wavelength of 357.8 nm, is proportional to the Cr_2O_3 concentration in the sample. Particle size distribution of each starch source was determined by dry sieve analysis. For this measurement, 100 g material was put on top of a set of 7 sieves: 3.15, 2.5, 2.0, 1.4, 1.0, 0.6, and 0.2 mm. Sieves were vibrated with an amplitude of 2 mm for 4 min (with interruptions) and the weight of residues on top of each sieve was determined.

Calculations

The following variables were calculated in three segments of the small intestine: digestion coefficient of starch (DC_S), absorption coefficient of glucose (AC_G), digestion coefficient of starch free dry matter and mean retention time (MRT). The formula used to calculate the digestion coefficient of starch was as follows:

$$DC_S = 1 - \left[\frac{(\text{starch}/Cr_2O_3)_{\text{digesta}}}{(\text{starch}/Cr_2O_3)_{\text{diet}}}\right]$$

where DC_S is the digestion coefficient of starch (starch and Cr_2O_3 in g/kg).

Starch was calculated as (total glucose – free glucose) x 0.9. A theoretical glucose absorption was calculated similarly. For this calculation, starch in the numerator was thus replaced by total glucose multiplied by the factor 0.9. It was assumed that all free glucose in digesta and excreta originated from starch. With this assumption, total glucose was related to the Cr_2O_3 content. Collected excreta from the last 2 d of the experimental period were used to calculate total starch digestion. DC_S values for common beans, peas, horse beans and potato starch were calculated from DC_S values of diets E, H, I and K and DC_S values of maize (hammer milled, diet B) which were corrected for differences in MRT. MRT was calculated using the following formula:

$$\mathrm{MRT} = \frac{1440 \cdot \mathrm{C} \cdot \mathrm{W}}{\mathrm{I}}$$

where MRT is the mean retention time (min), *C* is the Cr_2O_3 concentration in the digesta (mg/g), *W* is the weight of dry gut contents (g), *I* is the Cr_2O_3 intake over 24 h (mg feed intake · Cr_2O_3 content in feed) and 1440 equals min/d.

By relating the digestion coefficients to the mean retention time, starch digestion rate was calculated. MRT in the duodenum and rectum was not measured. On the basis of results of previous experiments at our institute and in the literature (Shires, 1987, Van der Klis et al., 1990), MRT in the duodenum was assumed to be 5 min and MRT in the rectum was assumed to be 20 min for all diets. We assumed that starch digestion did not occur prior to the small intestine. The shape of the digestion curve was assumed to follow first-order kinetics and the following equation was used to estimate the digestibility characteristics:

$$DC_t = D \cdot (1-e^{-k(d) \cdot t}),$$

where DC_t is the part of ingested starch digested at time *t*. Fraction *D* is the asymptote, which is the potentially digestible starch fraction that will digest at a rate of k_d (/h). A k_d-value of 2.00 means a starch digestion rate of 200%/h. The Marquardt method of the PROC NLIN procedure, an iterative curve-fitting procedure (SAS Institute Inc., 1989), was used to reduce the residual sums of squares associated with the regression model.

Statistical analysis

Before statistical analysis, the digestion coefficients were transformed by the logit transformation {ln [p/(100–p)]} to meet statistical assumptions (normal distribution and homogeneity of variance). Differences in DC_S and MRT were tested according to the following statistical model:

$$Y_{ijkl} = \mu + P_i + F_j + SS_k + e_{ijkl}$$

where Y_{ijkl} equals DC_S or MRT, μ is the overall mean, P_i is the period effect (i = 1, 2), F_j is the floor effect (j = 1, 2, 3), SS_k is the effect of starch source (k = 1, ..., 12) and e_{ijkl} is the error term.

The effects were tested using the general linear model of the SAS package (SAS Institute Inc., 1989). Differences were considered significant when $P < 0.05$. Mean comparisons were made using the least-square means option and least-square means were transformed back to the original scale for presentation.

Results

Differences among starch sources were found in each segment, but they were most pronounced in the upper small intestine (Table 3). Starch digestion occurred mainly in the upper small intestine. On average, 90% of digested starch in cereal grains was digested before the ileum and 98% before the posterior ileum. These values were lower for common beans (50 and 87%, respectively), peas (71 and 91%, respectively), horse beans (70 and 92%, respectively) and potato starch (60 and 77%, respectively). A large difference indicates a gradual starch digestion along the small intestine. Tapioca starch digestion was almost complete in the upper small intestine. A substantial proportion of ingested starch from peas and beans was digested in the ileum (23-36%). A smaller proportion of potato starch (13%) and cereal starch (6-13%) was digested in this part of the small intestine.

Table 3. Digestion coefficients of starch in different segments of the small intestine of broiler chickens fed diets containing different starch sources[1,2].

Starch source	Posterior jejunum	Anterior ileum	Posterior ileum	Total tract
		Ingested starch, %		
Wheat	88.2^{ef}	92.9^{d}	94.4^{d}	93.8^{d}
Maize, hammer-milled	88.8^{fg}	95.3^{e}	96.9^{ef}	97.4f^{g}
Maize, roller-milled	91.3^{g}	96.6^{f}	97.4^{f}	97.7^{g}
Maize, waxy	83.9^{d}	94.3^{de}	96.6^{e}	97.2^{f}
Common beans, heat-treated	36.1^{b}	62.9^{b}	72.3^{b}	74.5^{b}
Barley	89.8f^{g}	97.3^{g}	98.1^{g}	98.3^{h}
Sorghum	83.7^{d}	93.0^{d}	95.3^{d}	95.4^{e}
Peas	57.4^{c}	73.0^{c}	80.4^{c}	81.0^{c}
Horse beans	57.0^{c}	74.8^{c}	81.5^{c}	81.5^{c}
Tapioca pellets	97.7^{h}	98.7^{h}	98.9^{h}	98.9^{i}
Raw potato starch	19.8^{a}	25.3^{a}	32.9^{a}	31.7^{a}
Brown rice, not polished	85.1^{de}	94.8^{e}	96.8^{e}	97.3^{f}
Pooled SD	4.2	2.7	1.7	1.6

[1] n = 6 experimental units per treatment. An experimental unit was a pen containing 10 broiler chickens.

[2] Means in a column without a common superscript differ, $P < 0.05$.

Ileal starch digestibility (represented by DC_S in the PI) was not complete. Undigested starch at the PI varied from 1 (tapioca) to 67% (potato starch). This starch fraction was not fermented in the hind gut because DC_S in the total tract was not higher than in the PI. Waxy maize, sorghum and rice had a low starch digestion coefficient in the upper small intestine compared with other cereals. This difference became less in the lower parts of the small intestine. Starch digestion along the small intestine was similar for hammer-milled and roller-milled maize. Peas and horse beans showed a similar starch digestion along the small intestine as well. The ranking of feedstuffs according to DC_S was the same in the AI and PI, but not in the PJ.

Differences in potential starch digestibility (D) between feedstuffs (Table 4) were similar to those in ileal starch digestibility (Table 3). Fractional starch digestion rates (k_d) differed considerably among feedstuffs. It is clear that a low extent of starch digestion is combined with a slow starch digestion. Total mean retention time (MRT) in the jejunum and ileum (Table 5) varied from 136 min (barley diet) to 182 min (potato starch diet). MRT in the AJ was considerably shorter than in the three following segments. DC_S and MRT were negatively correlated (PJ: $r = -0.76$; AI: $r = -0.74$; PI: $r = -0.57$; $n = 12$). The MRT of the diet with roller-milled maize tended to be shorter than that of the diet with hammer-milled maize ($P = 0.11$).

Table 4. Starch digestion characteristics in the small intestine of broiler chickens fed diets containing different starch sources[1,2].

Starch source	Potential starch digestibility (D)	Fractional starch digestion rate (k_d)
	%	/h
Wheat	93.9 ± 0.42	2.51^{d} ± 0.127
Maize, hammer-milled	96.8 ± 0.38	2.55^{d} ± 0.093
Maize, roller-milled	97.4 ± 0.40	3.13^{e} ± 0.138
Maize, waxy	96.9 ± 0.54	1.93^{c} ± 0.072
Common beans, heat-treated	97.0 ± 8.96	0.46^{a} ± 0.082
Barley	98.5 ± 0.58	2.51^{d} ± 0.129
Sorghum	95.4 ± 0.47	1.81^{c} ± 0.063
Peas	85.3 ± 2.19	1.03^{b} ± 0.089
Horse beans	85.6 ± 2.03	0.98^{b} ± 0.081
Tapioca pellets	98.9 ± 0.06	4.29^{f} ± 0.094
Raw potato starch	38.4 ± 4.31	0.55^{a} ± 0.137
Brown rice, not polished	97.1 ± 0.39	2.00^{c} ± 0.057

[1] Values are means ± SE, $n = 6$ experimental units per treatment. An experimental unit was a pen containing 10 broiler chickens. Means in a column without a common superscript differ, $P < 0.05$.

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

Absorption coefficients of glucose (AC_G) were also calculated (data not given). AC_G showed a pattern similar to DC_G in Table 3. Glucose from tapioca starch was immediately absorbed after digestion. In the PJ, 4-5% of starch from most of the examined feedstuffs was not absorbed after digestion.

Table 5. Mean retention time (MRT) in the small intestine of broiler chickens fed diets containing different starch sources[1,2].

Starch source	Anterior jejunum	Posterior jejunum	Anterior ileum	Posterior ileum	Jejunum + ileum
			min		
Wheat	21^{e}	43^{bcde}	42^{ab}	51^{abc}	157^{cd}
Maize, hammer-milled	16^{bc}	39^{abc}	46^{abc}	52^{abc}	153^{bc}
Maize, roller-milled	13^{ab}	35^{a}	41^{a}	51^{abc}	140^{ab}
Maize, waxy	18^{cde}	41^{bcd}	50^{cd}	58^{bcd}	167^{cde}
Common beans, heat-treated	19^{cde}	45^{def}	52^{cd}	56^{bcd}	172de
Barley	11^{a}	42^{bcd}	39^{a}	43^{a}	136^{a}
Sorghum	18^{cde}	49^{ef}	52^{cd}	60^{cd}	178^{e}
Peas	18^{cde}	44^{cde}	43^{ab}	52^{abc}	156^{bcd}
Horse beans	17^{cd}	49^{ef}	53^{d}	51^{ab}	169^{cde}
Tapioca pellets	20de	38^{ab}	41^{a}	56^{bcd}	154^{bc}
Raw potato starch	26^{f}	50^{f}	53^{cd}	53^{bc}	182^{e}
Brown rice, not polished	19^{cde}	40^{abcd}	48^{bcd}	63^{d}	171de
pooled SD	3.4	5.3	6.2	8.4	13.7

[1] n = 6 experimental units per treatment. An experimental unit was a pen containing 10 broiler chickens.
[2] Means in a column without a common superscript differ, $P < 0.05$.

Of common bean starch, 9% was not absorbed in this segment after digestion. Similar differences between common bean starch and starch from other feedstuffs were observed in the AI and PI. Ileal digestibility of starch-free dry matter varied from 45 (barley diet) to 56% (horse bean diet) (data not shown). Starch-free dry matter was not further degraded in the hind gut.

Mean particle size varied from 0.3 (potato starch) to 1.7 mm (roller-milled maize). Most starch sources had a mean particle size between 0.8 and 1.2 mm (Table 6).

Table 6. Particle size distribution and mean particle size (MPS) of starch sources in experimental diets.

Starch source	Fraction			
	< 0.6 mm	0.6 - 1.4 mm	>1.4 mm	MPS
		%		*mm*
Wheat	33	46	22	0.94
Maize, hammer-milled	42	48	10	0.77
Maize, roller-milled	7	19	74	1.68
Maize, waxy	30	45	25	0.99
Common beans, heat-treated	42	43	15	0.81
Barley	25	49	25	1.06
Sorghum	39	52	10	0.80
Peas	39	51	11	0.80
Horse beans	21	45	35	1.19
Tapioca pellets	70	26	5	0.51
Raw potato starch	87	9	4	0.29
Brown rice, not polished	69	29	2	0.51

Discussion

Digestion of the starch found in different feedstuffs was measured in this experiment. The feedstuffs not only differed in starch characteristics, but also in other aspects. In most feedstuffs, starch granules are found in the endosperm. They are embedded within a protein matrix (McAllister et al., 1993) and shielded by cell walls (Eastwood, 1992). These physical structures most likely affect starch digestion, even after milling. We intended to maintain these protective structures because they are also found in practical broiler diets. Because digestion of starch can occur throughout the small intestine, a variation in rate within that compartment will have an effect on glucose absorption. It may be argued that it is important to have a continuous supply of glucose throughout the day rather than sharp peaks. A continuous glucose supply results in a gradual insulin release and may lead to a more efficient utilization of amino acids because glucogenic energy should be available for protein deposition to occur.

This experiment clearly illustrated that for most feedstuffs, starch digestibility is high, but incomplete at the end of the ileum. The undigested starch fraction in the starch sources studied varied from 1% for tapioca pellets to 67% for raw potato starch. Undigested starch fractions of cereal grains and legume grains were between these two extremes. Cereal grains had an undigested starch fraction between 2 and 6%. In legume grains, this fraction varied from 19 to 28%. Undigested starch may serve as a substrate for bacteria present in the hind gut. However, starch fermentation is energetically less efficient than enzymatic starch digestion in the small intestine (Dierick et al., 1989). In our experiment, total starch digestion (measured in excreta) was the same as ileal starch digestion, indicating that the undigested starch fraction was not fermented in the hind gut. This observation is consistent with the observation that the undigested starch fraction is similar for conventional and germ-free chicks (Kussaibati et al., 1982). Short-chain fatty acid production has been observed in the broiler intestinal tract, mainly in the ceca (Choct et al., 1996), implying fermentation of carbohydrates. In our experiment, starch digestion was calculated from remaining starch in the digesta, but fermentation of starch by microbes cannot be excluded. The differences in the undigested starch fractions among feedstuffs can be explained in part by the starch characteristics of the feedstuffs. Starch in cereal grains has an A-type structure, starch in legume grains and tapioca has a C-type structure and starch in potatoes has a B-type structure (Zobel, 1988). Amylose to amylopectin ratios are highest in legume grains (± 0.33), followed by cereal grains (± 0.25) (except rice) and are lowest in tapioca, potatoes and rice (± 0.20) (Eliasson and Gudmundsson, 1996). For some cereal grains genotypes exist with higher (high amylose varieties) or lower (waxy varieties) amylose contents. Finally, starch granules in potatoes are larger than in most cereals and tapioca (Eliasson and Gudmundsson, 1996). We assume that these distinctions in starch characteristics also applied to the batches used in this experiment.

Estimated potential starch digestibility *D* (Table 4) was similar to actual ileal starch digestion (Table 3) for cereal grains and tapioca. It appears that the undigested starch fraction in these feedstuffs is truly indigestible. However, in the case of legume grains and potato starch, estimated potential starch digestibility (*D*) was higher than ileal starch digestion. Apparently, digestion of potentially digestible starch was incomplete due to a combination of a slow starch digestion rate in these feedstuffs and the relatively short retention time in the gastrointestinal tract of broiler chickens.

Starch digestion in the upper small intestine was greatest for tapioca pellets, which had a very fine particle size distribution (Table 6). Thus, the relative surface area was large. Furthermore, the pelleting process may have gelatinized part of the starch in the product (Thomas, 1998). Digestion coefficients in the AI showed the same ranking among feedstuffs as in the PI. The ranking of feedstuffs based on digestion coefficients in the PJ was different from that in the ileum. Waxy maize, sorghum and rice starch were slow starters compared with starch in other cereals. Wheat starch was digested to the same extent as barley starch in the PJ. Tapioca starch was digested very rapidly (98% was digested before the ileum). The SD calculated indicated that starch digestion coefficients within feedstuffs were less variable in the posterior parts of the small intestine compared with the anterior parts. AC_G is the proportion of ingested starch that has been theoretically absorbed in the form of glucose. The difference between DC_S and AC_G was most pronounced for common beans in all segments of the small intestine. The slow digestion of starch in this feedstuff (Table 4) ensures a constant release of glucose. When the majority of a starch is digested in the anterior parts of the small intestine, then most glucose will already be absorbed before the digesta reaches the more posterior parts. Another explanation may be that the absorptive capacity of the small intestine was reduced due to damage to the intestinal wall caused by lectins (Jaffé, 1980).

The negative correlation between DC_S and MRT indicates that feedstuffs with a low starch digestion coefficient stay longer in the small intestine. Starch digestion data from Table 3 are end points that are determined by starch digestion rate and MRT. Thus, the site of starch digestion is not an accurate indicator of starch digestion rate because passage rate is also affected by diet.

Starch digestion rates varied considerably among feedstuffs. Common beans and raw potato starch had an extremely slow starch digestion (k_d: 0.5 /h). Starch from horse beans and peas was also digested slowly (k_d: 1.0 /h). This slow digestion is undoubtedly associated with the incomplete starch digestion in the birds. Waxy maize, sorghum and rice displayed a gradual starch digestion (k_d: 1.8-2.0 /h), whereas wheat, barley and hammer milled maize showed a rapid starch digestion (k_d: 2.5 /h). Roller-milled maize (k_d: 3.1 /h) and tapioca pellets (k_d: 4.3 /h) had extremely high starch digestion rates. No difference in starch digestion rates for wheat, barley and hammer-milled maize was observed. Wheat starch, however, was digested to a lesser extent. Apparently wheat contained a larger indigestible starch fraction.

Different milling treatments did not affect ileal starch digestibility of maize. In both cases, starch digestibility was 97%. Site of starch digestion also was not different for these two treatments (Table 3). The starch digestion rate of roller-milled maize, however, was higher than that of hammer-milled maize. On the basis of the differences in mean particle size (Table 6), a more rapid starch digestion was expected for hammer-milled maize. It is possible that the two milling treatments of maize not only resulted in different particle size distributions, but also changed other particle properties (shape of particles), thereby affecting feed passage rate (see Table 5) and starch digestion rate.

We conclude that ileal starch digestion varies considerably among different feedstuffs. Most of these differences originate in the upper small intestine. Ileal starch digestibility is therefore related to starch digestion rate. Some feedstuffs have the same ileal starch digestion, but differ in starch digestion rate. Other feedstuffs have the same starch digestion rate, but differ in ileal starch digestion. For most feedstuffs ileal starch digestion was high, but incomplete, and no further starch digestion occurred in the hind gut. This experiment shows that the site of starch digestion is not an accurate indicator of starch digestion rate. Starch digestion rate varies among native starches, as present in the feedstuffs. This provides the opportunity to manipulate the availability of glucose throughout the day. The practical relevance of starch digestion rate in broiler nutrition has to be established.

Chapter 4

In vitro Starch Digestion Correlates Well with Rate and Extent of Starch Digestion in Broiler Chickens

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Abstract

Current feed evaluation systems for poultry are based on digested components (fat, protein and nitrogen-free extract). Digestible starch is the most important energy source in broiler chicken feeds and is part of the nitrogen-free extract fraction. Digestible starch may be predicted using an in vitro method that mimics the digestive process in the gastrointestinal tract of broiler chickens. An experiment was designed to use this method for predicting site, rate and extent of starch digestion in broiler chickens. In vitro starch digestion was studied in 12 experimental diets differing in starch sources. These diets were also used in a digestibility trial with broiler chickens. Correlations between in vitro and in vivo starch digestion were calculated. Starch digestion after 2 h incubation correlated well with in vivo starch digestion in the first half of the small intestine (r = 0.94). A 4-h incubation period resulted in a good correlation between in vitro starch digestion and ileal starch digestion (r = 0.96). In vitro starch digestion rate (/h) correlated well with in vivo starch digestion rate (r = 0.87). In vitro starch digestion of individual starch sources was additive. It appeared that legume seeds and waxy maize contained two starch fractions, which were digested at different rates. We concluded that starch digestion rate in broiler chickens is well predicted by the in vitro method.

Keywords: starch, *in vitro*, digestion rate, broiler chickens

Introduction

Starch provides > 50% of the apparent metabolizable energy (AME) in broiler feeds. The Dutch feed industry (Centraal Veevoederbureau, 1999) evaluates feedstuffs on the basis of digested components (fat, protein and nitrogen-free extract). Starch is part of the nitrogen-free extract (NFE) fraction. The AME content of wheat is affected by starch digestibility (Rogel et al., 1987, Wiseman et al., 2000). Undoubtedly, this also applies to other feedstuffs containing starch. Starch digestibility is affected by intrinsic factors such as starch structure and composition (Oates, 1997) and associations between starch granules and protein and cell wall structures within the feedstuff (Eastwood, 1992). Furthermore, extrinsic factors such as processing of starch sources and conditions in the gastrointestinal tract also affect starch digestibility. Soluble non starch polysaccharides in the diet increase gut viscosity, which impairs starch digestion (Classen, 1996, Refstie et al., 1999). Finally, passage rate of digesta in the small intestine determines the time available for starch hydrolysis.

Englyst et al. (1982) introduced the term Resistant Starch (RS) in 1982; the term was later defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Asp, 1992). This RS fraction was further subdivided into physically inaccessible starch (RS_1), resistant starch granules (RS_2) and retrograded amylose (RS_3). Results from Weurding et al. (2001) showed that starch digestion rate in the small intestine of broiler chickens varies considerably among feedstuffs. Digestion rate of the digestible starch fraction and the enzymatically resistant starch fraction will affect the extent of starch digestion. In human nutrition, the kinetics of starch digestion are already considered to be an important food characteristic. In 1981, Jenkins et al. (1981) introduced the glycemic index, which reflects the effect of glucose absorption rate on plasma glucose levels. Englyst et al. (1996) showed that starch digestion rate correlates well with glycemic index. These two traits can be used for managing diabetes, sports performance and appetite research (Brand-Miller, 1999). Starch digestion rate may also be important in broiler nutrition because it may affect plasma insulin levels and the availability of nutrients at a specific time (synchronization of energy and amino acid digestion). This may have an effect on the efficiency of protein deposition in the broiler chicken, which is of economic interest to the poultry farmer.

If starch digestion rate turns out to be an important trait for broiler chickens, then a reliable, rapid and inexpensive laboratory analysis to predict this trait is warranted. Englyst et al. (1992) proposed an *in vitro* method that simulates starch digestion in the small intestine of humans. On the basis of this *in vitro* method, he fractionated starch in rapidly digestible starch (RDS), slowly digestible starch (SDS) and RS. The objective of this experiment was to investigate whether a modified version of the *in vitro* starch digestion method proposed by Englyst et al. (1992) can be used to predict site, rate and extent of starch digestion in broiler chickens.

Materials and methods

Experimental diets and feedstuffs

In vitro starch digestion of 12 experimental diets was measured in a laboratory experiment. Diet composition and starch digestion in three different segments of the small intestine is given by Weurding et al. (2001). Eight diets contained only one starch source and four diets contained two starch sources. *In vitro* starch digestion was also determined in each of the 12 starch sources. The 12 starch sources are given in Table 1.

In vitro procedure

In vitro starch digestion was determined using a modified version of the method described by Englyst et al. (1992). This modified *in vitro* method simulates digestive behavior in the alimentary tract of broilers. In contrast to Englyst et al.

(1992), diets and feedstuffs were milled in a Retsch mill to pass a 1-mm screen, thus simulating grinding action in the gizzard.

Table 1. *In vitro* starch digestion coefficients (DC) of experimental diets with different starch sources after several incubation times.

Diet	Incubation time (h) 0.25	0.5	0.75	1	2	3	4	5	6
	% of total starch								
Wheat	28.8	51.8	71.0	84.1	97.8	98.3	99.3	98.9	98.8
Maize, hammer-milled	28.4	47.1	61.2	71.7	91.3	96.6	98.6	99.0	99.6
Maize, roller-milled	29.7	48.8	63.6	74.6	95.3	100.5	102.7	103.3	104.2
Maize, waxy	26.7	44.3	57.8	67.6	88.1	94.0	96.2	98.7	99.1
Common beans[1]	24.6	37.4	45.9	51.8	69.5	81.0	86.5	92.2	96.7
Barley	25.5	44.3	60.8	73.2	92.8	99.3	99.7	100.4	101.6
Sorghum	19.4	38.1	55.7	69.4	95.0	99.5	102.3	102.8	103.7
Peas[1]	15.7	27.8	37.6	45.8	67.0	77.1	85.1	91.9	94.6
Horsebeans[1]	13.2	19.2	35.0	38.4	58.0	70.4	77.7	83.4	85.9
Tapioca	72.8	85.7	89.9	92.1	96.0	96.9	97.4	97.5	97.5
Potato starch[1]	11.8	15.7	19.2	22.4	33.9	44.1	53.8	60.8	66.5
Brown rice	27.3	46.9	62.0	72.2	91.5	96.5	98.7	98.9	99.0
SD	2.55	3.09	1.56	1.49	1.59	2.75	2.01	1.88	1.26

$n = 2$.

[1] Calculated values: $DC_{\text{experimental starch source in diet}} = (DC_{\text{diet}} - a \times DC_{\text{maize}}) / b$; a and b are starch fractions provided by maize and the experimental starch source, respectively.

Test tubes containing feed sample, glass balls and a pepsin-HCl solution were incubated in a water bath (37°C) for 30 min to simulate passage through the proventriculus. After this pre-incubation, the procedure described by Englyst et al. (1992) was carried out. Buffer solution and an enzyme cocktail were added and tubes were placed horizontally in a shaking water bath (37°C) for 0.25, 0.50, 0.75, 1, 2, 3, 4, 5 and 6 hours. After each incubation time, aliquots were taken from the tubes and the amount of released glucose was measured colorimetrically according to a glucose oxidase method (Glucose oxidase diagnostic kit 166391, Boehringer Mannheim, Mannheim, Germany). We used nine incubation times instead of the two (20 and 120 min) used by Englyst et al. (1992). This was done to improve the estimation of starch digestion rate. The four incubation times during hour 1 represent the steep part of the digestion curve. A good estimation of starch digestion rate requires sufficient measurements in this part of the curve. For most feedstuffs, the digestion curve will have reached the asymptotic level after 6 hours. *In vitro* starch digestion was determined separately for the experimental diets and the starch sources. Four analyses were performed. Each analysis contained either 12 different experimental diets or 12 different starch sources. Total starch (TS) and free glucose (FG) content was determined as described for a finely divided sample by Englyst et al. (1992). Digestion coefficient of starch at time t (DC_t) was calculated as follows:

$$[(G_t - FG) \cdot 0.9] / TS, \tag{1}$$

where G_t represents the amount of glucose present at time *t*. Starch digestion coefficients of common beans, peas, horsebeans and potato starch in both *in vivo* and *in vitro* experiments were calculated from digestion coefficients of the total diet and maize (Weurding et al., 2001). It was assumed that starch digestion coefficients in compound feeds are additive. Preliminary data suggested that *in vitro* starch digestion follows first-order kinetics and *in vitro* starch digestion rate was estimated using the following equation:

$$DC_t = D \cdot (1-e^{-k(d) \cdot t}) \text{ with } D \leq 100 \qquad (2)$$

where DC_t is the starch fraction digested at time *t*, fraction *D* is the potentially digestible starch fraction that will digest at a fractional rate of k_d (/h). This terminology is similar to that used for protein degradation in the rumen of dairy cows (Ørskov and McDonald, 1979). The Marquardt method of the PROC NLIN procedure (an iterative curve fitting procedure) (SAS Institute Inc., 1989) was used to reduce the residual sums of squares associated with the regression model. *In vitro* starch digestion at each incubation time was correlated with *in vivo* starch digestion at the different sites of the small intestine [posterior jejunum (PJ), anterior ileum (AI) and posterior ileum(PI)] and to total starch digestion as determined by Weurding et al. (2001). The incubation times for which *in vitro* starch digestion showed the best correlation with *in vivo* starch digestion in the PJ and PI were determined. *In vivo* and *in vitro* estimates for potential starch digestibility (*D*) and fractional digestion rate (k_d) were compared using regression analysis. Relations between *in vitro* and *in vivo* starch digestion were investigated by regressing *in vivo* on *in vitro*. The influence of a specific treatment on the regression equation was investigated by calculating the leverage of each treatment (a measure for the relative position of an observation in relation to the other observations). Treatments that have a high leverage (> $5/n$, in which *n* is the number of observations) are outside the range of the x-axis (Oude Voshaar, 1995). When leverage was high, the influence of that specific treatment on the regression equation was studied.

Additivity of in vitro starch digestion

Additivity of *in vitro* starch digestion was tested by comparing *in vitro* starch digestion of diets containing two different starch sources with that of the separate measurements of the two starch sources ($P < 0.05$). Additivity was tested using a *t* test, which tested $a \cdot DC_{maize} + b \cdot DC_x - DC_y = 0$, where *DC* is the starch digestion coefficient, *x* is the experimental starch source and *y* is the experimental diet containing maize and *x* in proportions *a* and *b* respectively. The effect of other feed ingredients on starch digestion was also studied by comparing starch digestion in individual starch sources and in the corresponding experimental diets with only one starch source (8 diets). A *t* test was used, which tested $DC_{feedstuff} - DC_{diet} = 0$. Prior to both *t* tests, homogeneity of variance was tested.

Goodness of fit of starch digestion curves
Predicted starch digestion values (based on equation 2) were compared with observed starch digestion values. If alternating periods of underestimation and overestimation of starch digestion were observed (resulting in a systematic pattern in the plot of residuals), the curve fitting procedure was repeated using a two-phase model:

$$DC_t = D_1 \cdot (1-e^{-k(d)1 \cdot t}) + D_2 \cdot (1-e^{-k(d)2 \cdot t}) \text{ with } D_1 + D_2 \leq 100 \qquad (3)$$

An *F*-test as described by Motulsky and Ramsnas (1987) was used to determine whether the two phase model gave a significant improvement of the fit ($P < 0.05$).

$$F = [(SS_1 - SS_2) / (df_1 - df_2)] / (SS_2 / df_2)$$

where F is the F-value for the comparison of both curves; SS_1 is the sum of squares of fit for the one-phase model; SS_2 is the sum of squares of fit for the two-phase model; df_1 represents the degrees of freedom for the one-phase model; and df_2 represents the degrees of freedom for the two-phase model.

Results

In vitro starch digestion differed among the diets (Table 1). Potato starch and legumes displayed lower starch digestion values than cereal grains and tapioca. Sorghum had lower starch digestion values within the first 30 min compared with the other cereal grains. *In vitro* and *in vivo* starch digestion (PJ) differed in a few aspects. *In vitro* starch digestion (2 - 6 h) of common beans was similar to that of peas and higher than horsebeans. *In vivo* common bean starch digestion however, was much lower than pea and horsebean starch digestion. Ileal starch digestion of wheat and sorghum was low compared with that of other cereal grains. *In vitro* starch digestion for these cereals was similar to that of the other cereals. Table 2 presents correlation coefficients for *in vitro* starch digestion at different incubation times and *in vivo* starch digestion at different segments of the gastrointestinal tract of broilers. After 2 h of incubation, *in vitro* starch digestion showed the best correlation with *in vivo* starch digestion in the PJ, (r = 0.94). Starch digestion at the AI was best predicted after 3 h of incubation (r = 0.96). Starch digestion at the PI was best predicted by a 4-h incubation period (r = 0.96). Figure 1a and b show the relation between *in vitro* starch digestion and starch digestion in the PJ and PI. *In vivo* starch digestion coefficients at the PJ and PI can be predicted from these regression equations:

$$DC_{PJ} = 1.1649 \times DC_{2h} - 21.489 \qquad R^2 = 0.8804 \qquad \text{SEM} = 9.00 \quad (4)$$
$$DC_{PI} = 1.2862 \times DC_{4h} - 30.863 \qquad R^2 = 0.9153 \qquad \text{SEM} = 5.82 \quad (5)$$

Table 2. Correlation matrix showing correlation coefficients between *in vivo* starch digestion at different sites of the broiler chicken alimentary tract and *in vitro* starch digestion after several incubation times[1].

	Sampling site in the alimentary tract			
In vitro incubation time, h	Posterior jejunum	Anterior ileum	Posterior ileum	Excreta
		r (x 100)		
0.25	56	49	46	46
0.5	75	69	66	65
0.75	88	84	81	81
1	92	89	86	85
2	94	95	93	93
3	92	96	95	95
4	90	96	96	96
5	85	94	95	96
6	80	90	92	93

[1] Correlation coefficients > 58 are significant, $P < 0.05$.

Potato starch was at the outer reach of the *x*-axis in both figures and had a leverage of $7/n$ and $9/n$ in Figures 1a and b, respectively. Removing potato starch data from the dataset scarcely affected the prediction of starch digestion in the PJ. The correlation coefficient, however, was lowered to 0.88 after removing the potato starch data. Prediction of starch digestion at the PI, however, was affected when potato starch data were removed from the dataset. Scatter plots, in which mean values for the clusters of slowly (potato starch), gradually (legume grains) and rapidly digestible (cereal grains and tapioca) starch sources were used to show the correlation between *in vitro* and *in vivo* starch digestion, are presented in Figures 1c and d for PJ and PI, respectively.

Starch from tapioca was digested most rapidly (highest k_d), followed by cereal grains, legumes and finally potato starch (Table 3). Figure 2a and b shows correlations between *in vitro* and *in vivo* D and k_d. The potato starch data in Figure 2a were outside the range of the *x*-values (leverage = $9/n$). After removal of the potato starch data, no correlation existed between *in vivo* and *in vitro* D values. In Figure 2b, tapioca data were outside the range of the *x*-values (leverage = $11/n$). After removal of the tapioca data, a good correlation was found between *in vivo* and *in vitro* starch digestion rate (k_d).

Figure 3 shows predicted and observed *in vitro* starch digestion of diets with common beans, peas, horsebeans and potato starch. Data showed homogeneous variance. The difference between predicted and observed *in vitro* starch digestion was most pronounced for the potato diet (see Figure 3d). The legume diets (see Figs. 3a, b and c) showed less difference. Predicted and observed *in vitro* starch digestion of diets were not systematically different ($P > 0.05$). At each incubation time, differences between predicted and observed *in vitro* starch digestion in legume diets were < 10 units. Differences between predicted and observed *in vitro* starch digestion in the potato starch diet were up to 17 units.

When *in vitro* starch digestion data from waxy maize and legume grains (as measured in feedstuffs) were fitted to the one-phase model, alternating periods of underestimation and overestimation of starch digestion were observed. This is shown for horsebeans in Figure 4. The two-phase model that was used resulted in a better fit ($P < 0.05$). The D and k_d values for the two separated fractions of waxy maize and the legume grains are also presented in Table 3.

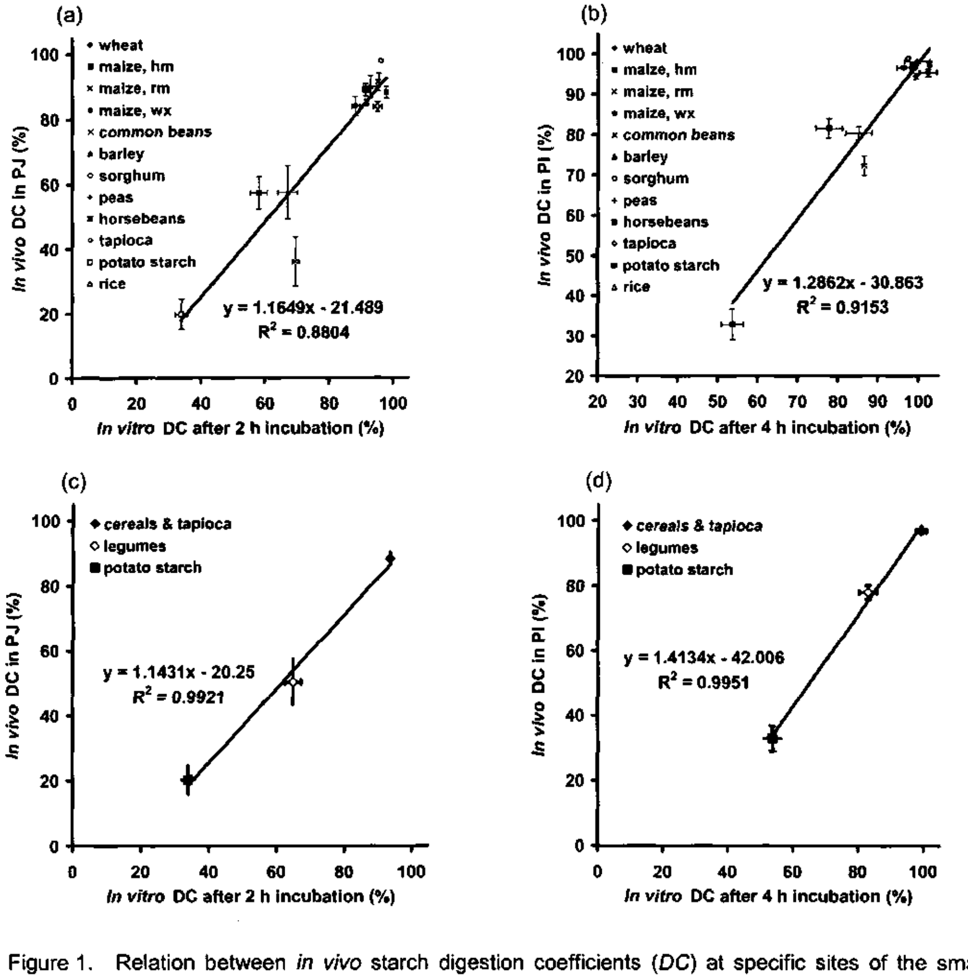

Figure 1. Relation between *in vivo* starch digestion coefficients (*DC*) at specific sites of the small intestine of broiler chickens (PJ, posterior jejunum: PI, posterior ileum) and *in vitro* starch digestion after 2 and 4 h of incubation. Panels a and b show mean values ± SD per feedstuff (hm, hammer-milled; rm, roller-milled; wx, waxy); panels c and d show mean values per group (see text). $n_{vivo} = 6$; $n_{vitro} = 2$.

Table 3. *In vitro* starch digestion characteristics of examined feedstuffs in experimental diets, calculated using the exponential curve equation $DC_t = D \cdot (1-e^{-k(d) \cdot t})$, where DC_t is proportion of starch digested at time t[1].

Feedstuff	Potential starch digestibility (D)	Fractional starch digestion rate (/h) (k_d)
	%	h^{-1}
As measured in diets		
Wheat	99.9 ± 0.93	1.59 ± 0.056
Maize, hammer-milled	99.1 ± 0.32	1.29 ± 0.015
Maize, roller-milled	100.0 ± 0.87	1.38 ± 0.043
Maize, waxy	97.9 ± 0.49	1.19 ± 0.021
Common beans, heat-treated	91.3 ± 2.09	0.88 ± 0.065
Barley	100.0 ± 0.86	1.25 ± 0.038
Sorghum	100.0 ± 1.59	1.11 ± 0.061
Peas	94.1 ± 1.49	0.65 ± 0.029
Horsebeans	87.6 ± 2.42	0.57 ± 0.043
Tapioca	95.5 ± 0.91	5.31 ± 0.359
Raw potato starch	73.8 ± 4.57	0.34 ± 0.043
Brown rice, not polished	99.0 ± 0.40	1.30 ± 0.019
As measured in feedstuffs		
Maize, waxy[2]		
phase 1	76.5 ± 8.51	1.12 ± 0.126
phase 2	23.0 ± 0.70	5.60 ± 2.373
Common beans, heat treated[2]		
phase 1	67.1 ± 1.37	0.40 ± 0.028
phase 2	13.3 ± 1.33	3.49 ± 0.759
Peas[2]		
phase 1	81.3 ± 0.77	0.34 ± 0.014
phase 2	12.1 ± 1.05	2.86 ± 0.389
Horsebeans[2]		
phase 1	73.7 ± 1.12	0.45 ± 0.027
phase 2	25.5 ± 1.25	7.18 ± 1.151

[1] Values are means ± SE, n = 2.
[2] Starch digestion characteristics of examined feedstuffs were calulated using a two-phase model $DC_t = D_1 \cdot (1-e^{-k(d)1 \cdot t}) + D_2 \cdot (1-e^{-k(d)2 \cdot t})$. These calculations were based on *in vitro* digestion data of the feedstuffs examined.

Discussion

Feedstuffs differ in more aspects than simply starch characteristics. Physical properties such as shielding structures (protein matrix, cell walls) may slow down starch digestion (Eastwood, 1992, McAllister et al., 1993). Feed milling is likely to reduce the protective effect of these physical structures and feed particles are further reduced in size by the grinding action in the gizzard of broiler chickens. For the *in vitro* technique, this process was mimicked by milling the diets over a 1-mm screen. The fact that *in vitro* and *in vivo* starch digestion correlated well suggests that milling simulated the grinding action in the gizzard well. The differences between feedstuffs for *in vitro* starch digestion were similar to the *in vivo* results (Weurding et al., 2001).

Tapioca starch was digested most rapidly, followed by cereal, legume and potato starch.

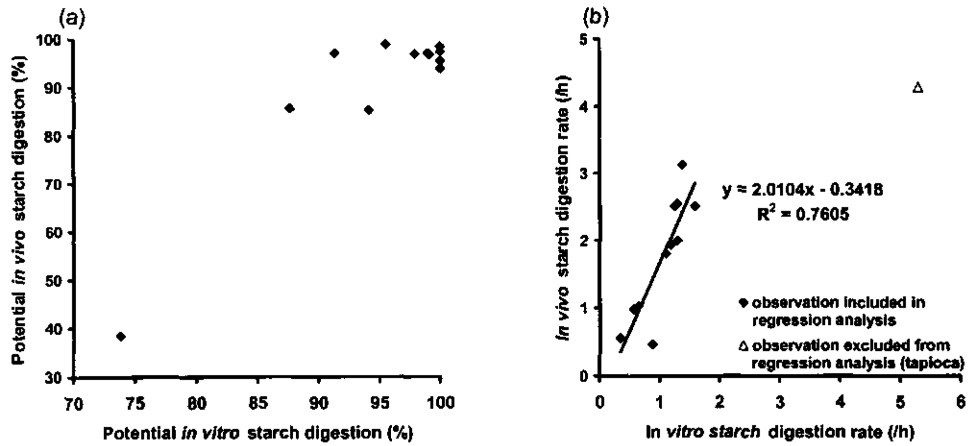

Figure 2. Relation between *in vivo* and *in vitro* starch digestion characteristics of 12 different starch sources for broiler chickens. n_{vivo} = 6; n_{vitro} = 2.

The *in vitro* results indicated that starch digestion up to the PJ can be predicted well after a 2-h incubation period. This fraction can be defined as rapidly digestible starch for poultry (RDS_p). It appears that for a prediction of ileal starch digestion, a 4-h incubation period is required. Slowly digestible starch for poultry (SDS_p) can be calculated from the difference between the starch fraction digested after 4 and 2 h of *in vitro* incubation. From the starch fraction that was not digested after 4 h of *in vitro* incubation, resistant starch for poultry (RS_p) can be derived. Englyst et al. (1992) defined RDS as starch digested after 20 min of *in vitro* incubation and SDS as starch that was digested between 20 and 120 min of *in vitro* incubation. RS is the starch fraction that was not digested after 120 min of *in vitro* incubation. Our definitions are not identical because we used different starch sources and processing methods compared with Englyst et al. (1992). Furthermore, there are differences in the gastrointestinal tract of broilers and humans.

Figure 1a and b show that more rapidly digestible starch sources than slowly digestible starch sources were used in the experiment. Starch in legume grains and potato starch was digested more slowly than starch in cereals and tapioca. From scatter plots in Figure 1a and b, it is clear that potato starch is located at the lower end of the *x*-axis. Omitting potato starch from the dataset scarcely affects the prediction of pre-ileal starch digestion. Omitting potato starch data from the regression analysis altered the regression line for prediction of ileal starch digestion. In that case, predicted *in vivo* values for slowly digestible starch sources were higher. To make the *in vitro* method applicable for a wide range of products, potato starch was included. Figure 1a and b clearly indicate three product groups, i.e., tapioca and cereal grains, legumes grains and potato starch. Figure 1c and d suggest that it is justifiable to keep potato starch in the dataset.

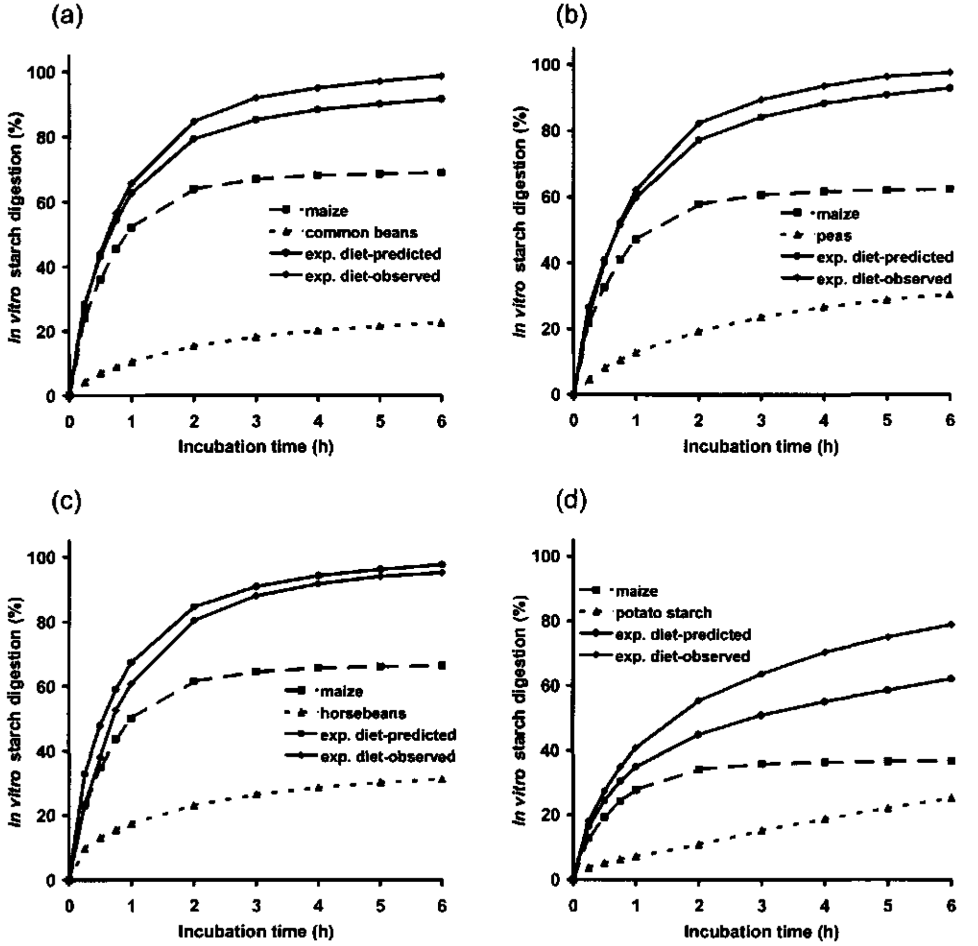

Figure 3. Predicted and observed *in vitro* starch digestion curves for experimental diets containing common beans (a), peas (b), horsebeans (c) and potato starch (d). The predicted curves are based on observed *in vitro* starch digestion curves for the individual feedstuffs: n = 2 observations per feedstuff and diet.

A distinction can be made between slowly (potato starch: 0.3 /h), gradually (legume grains: 0.6 - 0.9 /h), rapidly (cereal grains: 1.1 - 1.6 /h) and extremely rapidly (tapioca: 5.3 /h) digestible starch sources (Table 3). Starch digestion rate was higher *in vivo* than *in vitro* (Figure 2*b*). Tapioca was digested extremely quickly (both *in vivo* and *in vitro*). For tapioca, the starch digestion rate was lower *in vivo* than *in vitro*. This may be due to the absence of sufficient sampling sites in the anterior part of the small intestine (i.e., the steep part of the digestion curve). This may lead to an underestimation of *in vivo* starch digestion rate of rapidly digestible starch sources. We did not observe an interaction effect of starch sources on *in vitro* starch digestion ($P > 0.05$). Also, other feed components in the compound feeds did not affect *in vitro* starch digestion ($P > 0.05$). The potato starch diet showed the most pronounced difference between predicted and observed *in vitro* starch digestion. This is probably due to the fact that when potato starch is mixed with the buffer solution, it coagulates easily, which results in impaired starch digestion.

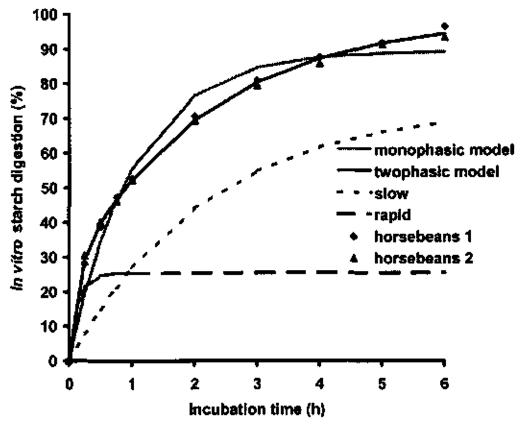

Figure 4. *In vitro* starch digestion of horsebeans ($n = 1$) fitted using a one or two-phase model. In the two-phase model, two fractions can be distinguished, i.e., a slowly and a rapidly digestible starch fraction.

The two-phase model gave a much better fit for *in vitro* starch digestion of legumes and waxy maize ($P < 0.05$). Apparently, two distinct different starch fractions with different digestion rates are present in these feedstuffs. It is not known whether this two-phase starch digestion is caused by different starch structures or by differences in accessibility to starch granules.

On the basis of the results of this experiment we conclude that our *in vitro* procedure can be used to predict both jejunal and ileal starch digestion as well as starch digestion rate. In turn, predicted ileal starch digestion can be used to improve the prediction of AME content (Rogel et al., 1987, Wiseman et al., 2000). Furthermore, Wiseman et al. (2000) found that wheat with rapidly digestible starch had a higher AME content. This suggests that a faster starch digestion rate results in a more efficient energy utilization by broilers. This may be related to the observation that a faster starch digestion rate results in a more complete starch digestion. This was seen *in vivo* (Weurding et al., 2001) as well as *in vitro*. *In vitro* *D*-values for rapidly digestible starch sources were between 96 and 100%. Slowly and gradually digestible starch sources had lower *in vitro* *D*-values. These observations seem to be inconsistent with observations reported by Truswell (1992). He reported no general correlation between glycemic index and the percentage of resistant starch in foods. Some foodstuffs he discussed, however, were heat-treated. These treatments result in starch gelatinization (increased starch digestion rate) and retrogradation (increased RS_3 fraction). Furthermore, the glycemic index is also affected by absorption of sugars other than glucose, whereas starch digestion rate affects only glucose absorption. Some of the feedstuffs discussed by Truswell contained high amounts of sugars compared with starch. It is of interest to know whether growth performance of broiler chickens is affected by iso-energetic diets with different starch digestion rates. A gradual starch digestion results in a more or less continuous availability of glucose. It is conceivable that ingested dietary protein will be utilized more efficiently with a continuous glucose supply. The response to insulin is also affected by glucose absorption rate (Truswell, 1992). Insulin is the major hormone that promotes anabolism in the body. It promotes the cellular uptake of amino acids and their incorporation into proteins (Fox, 1996).

In vitro digestion of sorghum starch started slowly compared with starch from other cereals, but after 2 h, it was higher than that of maize (both hammer-milled and waxy), rice and barley. After 15-min incubation, wheat starch digestion was similar to that of other cereals (except sorghum). It was, however, much higher after longer incubation times. Common bean starch digestion was similar to that of most cereals after 15 min of incubation. After longer incubation times, common bean starch digestion became more like that of pea starch. The observed differences between *in vivo* and *in vitro* digestion of wheat, sorghum and legumes (Table 1) may be due to the presence of antinutritional factors such as lectins, tannins and arabinoxylans. Their effect on the digestive process in the gastrointestinal tract is not simulated in the *in vitro* method. Other factors to which *in vitro* techniques do not respond, but which may affect starch digestion in birds, should also be noted. Effects of diets on passage rate and viscosity are not simulated at all. The *in vivo* trial (Weurding et al., 2001) revealed that diets containing substantial amounts of slowly digestible and resistant starch resulted in longer retention times in the small intestine. This may affect digestion coefficients. Furthermore, microbial fermentation of nutrients is not likely to occur *in vitro*. In addition, the *in vitro* technique implies that digestion products are not removed. Finally, there are no feedback mechanisms in the *in vitro* assay. There is an excessive amount of enzymes at the onset of the incubation period. On the other hand, a number of advantages of the *in vitro* method can be mentioned. The *in vitro* method is standardized, reliable, rapid and less expensive than *in vivo* measurements. There is no animal variation involved. Furthermore, the *in vitro* method enables simulation of many sites of the digestive tract of broiler chickens. Finally, *in vitro* methods are preferred in view of the welfare concerns related to animal experiments.

Chapter 5

The Effect of Site of Starch Digestion on Performance of Broiler Chickens

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Abstract

A growth trial with 420 broiler chickens (35 per experimental unit) was conducted in order to evaluate whether bird performance (d 0 to 38) is affected by site of starch digestion. Two diets were formulated with the same calculated apparent metabolisable energy-, digestible lysine- and digestible starch content. The diets contained starch sources with known amounts of rapidly digestible starch (RDS, starch digested until the posterior jejunum) and slowly digestible starch (SDS, starch digested in the ileum). Diets were either high (H diet) or low (L diet) in SDS content. RDS and SDS contents were 298 and 52 g/kg for the H diet and 345 and 7 g/kg for the L diet respectively. Birds receiving the H diet consumed more feed ($P < 0.10$), grew faster ($P < 0.01$) and had a lower feed conversion ratio ($P < 0.01$) than birds receiving the L diet. From the results it was concluded that broiler chickens perform better on diets containing a minimal amount of slowly digestible starch. Future experiments are necessary to investigate the relation between amount of slowly digestible starch and performance of broiler chickens.

Keywords: starch digestion, broiler chickens, performance

Introduction

More than 50% of the apparent metabolisable energy content of common diets for broiler chickens is provided by dietary starch. Starch is found as a storage carbohydrate in feedstuffs like cereal grains, legume seeds, tubers and roots. In unprocessed feedstuffs starch is present in granules (Banks and Muir, 1980). These granules can be disrupted by processing of the feedstuffs (Rooney and Pflugfelder, 1986). Native starches differ in granule size, amylose to amylopectin ratio and amylopectin structure (Eliasson and Gudmundsson, 1996). Differences in starch properties as well as differences in accessibility of the starch granules determine the susceptibility of starch to enzymatic degradation in the small intestine (Eastwood, 1992). Weurding et al. (2001) showed that site, rate and extent of starch digestion in the small intestine of broiler chickens differ considerably between a wide range of untreated feedstuffs. The extent of starch digestion in the small intestine of broiler chickens determines the amount of energy that is provided by dietary starch and this is positively correlated to the AME content of the diet (Rogel et al, 1987; Wiseman et al., 2000). There is a lack of information about the relation between the kinetics of starch digestion and bird performance. A rapid starch digestion may lead to the same extent of starch digestion as a gradual starch digestion, but the amount of starch digested at specific sites of the small intestine (jejunum and ileum) differs. Differences in site of starch digestion may have metabolic consequences that affect feed utilisation. The synchronisation of energy and protein digestion is affected by

starch and protein digestion rate. Glucose absorption rate affects insulin response (Björck et al., 2000), which may affect protein accretion (Fox, 1996). And the site of starch digestion may determine the site where it is utilised.
Starch-rich feedstuffs from the batches used in the digestibility trial by Weurding et al. (2001) were used in this trial. Therefore, feed formulation was based on known starch digestion coefficients at the posterior jejunum and posterior ileum. The objective of this experiment was to investigate whether site of starch digestion affects performance of broiler chickens.

Materials and methods

Animals and diets

The experiment was performed with 420 female Cobb 500 broiler chickens, housed in 12 floor pens. An experimental unit was formed by one pen, containing 35 birds. New-born chicks were assigned to one of two dietary treatments differing in starch sources (six pens per treatment). Another 420 birds from the same batch, which were not used in this experiment, were housed in the same building. The starch containing feedstuffs were from the same batches as used in a digestibility trial with broiler chickens (Weurding et al., 2001). These feedstuffs were stored at 4°C until the start of the experiment (one year). In both experiments, the feedstuffs were milled in a hammer mill over a 2.75 mm screen and diets were supplied as a mash. Therefore, digestion coefficients of starch at the posterior jejunum and at the posterior ileum of 29 d old broiler chickens were known for each starch source. Diets were formulated to have equal amounts of ileal digestible starch (IDS). Diets differed in amounts of rapidly digestible starch (RDS, starch digested until the posterior jejunum) and slowly digestible starch (SDS, starch digested in the ileum). Broiler chickens received a diet either high (H) in SDS (52 g/kg) or low (L) in SDS (7 g/kg). Both diets had the same amount of ileal digestible starch (350 g/kg). Total starch content differed between diets (380 vs. 361 g/kg). Composition and calculated energy and nutrient contents of diets given from d 0-30 are given in Table 1. From d 30-39 the animals received a diet without an anticoccidial.

Measurements and analysis

Weight and feed intake were measured in the starter (d 0-15), grower (d 15-30) and finisher (d 30-38) phase and feed conversion ratio (g feed / g weight gain) was calculated for each phase and the whole experimental period (d 0-38). Chemical analysis of diets included dry matter, ash, nitrogen (Dumas), crude fat, crude fibre and starch (Englyst et al., 1992). Milling over 2.75 mm will minimise differences in particle size, which could affect feed intake. Dry sieve analysis was performed to check whether particle size distributions were similar for both diets. For this measurement, 100 g material was put on top of a set of seven sieves: 3.15, 2.5, 2.0, 1.4, 1.0, 0.6 and

0.2 mm. Sieves were vibrated with an amplitude of 2 mm for 4 min (with interruptions) and weight of residues on top of each sieve was determined.

Differences in weight gain, feed intake and feed conversion ratio (FCR) between the two treatments were tested by means of the Student t-test.

Table 1. Composition and energy and nutrient contents of a diet with a high amount of slowly digestible starch (H) and a diet with a low amount of slowly digestible starch (L) (g/kg).

	H	L
Tapioca	-	487.5
Maize, roller milled	-	92.0
Maize, waxy variety	361.0	-
Peas	340.7	-
Sorghum	78.5	-
Soya bean oil	8.0	43.1
Animal fat	41.0	29.0
Potato protein	26.0	25.0
Meat meal	73.0	36.0
Fish meal	-	23.0
Feathermeal, hydrolysed	20.0	20.0
Soya beans, extracted	-	218.0
Sesameseed, expeller	24.7	-
Monocalcium phosphate	3.7	5.2
Sodium chloride	-	0.1
Vitamin-mineral premix[1]	10.0	10.0
Anticoccidial premix[2]	5.0	5.0
Acid insoluble ash (diamol)	-	4.0
L-Threonine	1.7	-
L-Lysine + DL-Methionine (20% + 10%)	-	2.1
L-Lysine + L-Tryptophan (18% + 5%)	6.7	-
Dry matter	877	890
ME (MJ / kg)[3]	12.66	12.66
Crude protein	206	196
Digestible lysine[3]	10.2	10.2
Digestible methionine + cysteine[3]	7.7	7.7
Rapidly digestible starch[3]	298	345
Slowly digestible starch[3]	52	7
Ileal digestible starch[3]	350	352
Total starch	380	361
Crude fat	84	87
Crude fibre	36	40
Ash	48	71

[1] This premix contained the following micro-elements (mg/kg): Mn, 6000; Zn, 3200; Fe, 4000; Cu, 1000; I, 80; Se, 10; retinol, 300; cholecalciferol, 5; α-tocopherol, 2500; menadione, 130; riboflavin, 400; pyridoxine, 100; cyanocobalamin, 1.5; nicotinic acid, 3000; folic acid, 50; d-pantothenic acid, 700; choline, 22000; biotin, 4; Avilamycin, 1000.

[2] This premix contained (mg/kg): meticlorpindol, 20000; methylbenzoquate, 1670.

[3] Calculated values.

Results

Particle size distributions

Diets had similar particle size distributions (Figure 1). Mean particle size was 0.70 and 0.69 mm for diets H and L respectively.

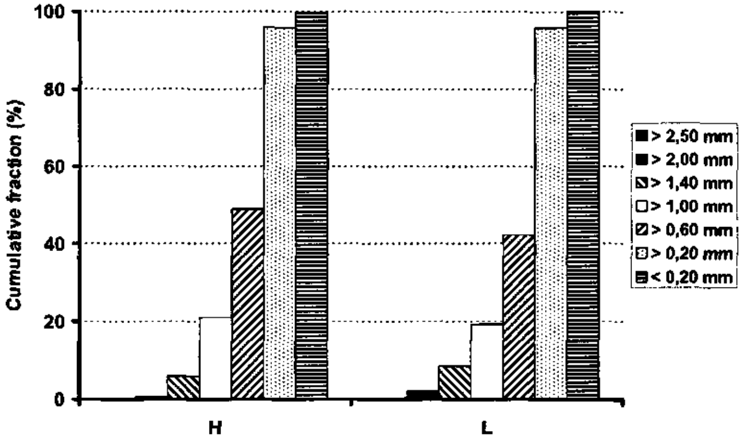

Figure 1. Cumulative particle size distribution of diets with either high (H) or low (L) amounts of slowly digestible starch.

Intake of starch fractions

Feed and starch intakes per day are presented in Table 2. Starch intakes are based on body weight (kg) and calculated as digestible starch intake, rapidly digestible starch intake and slowly digestible starch intake. Feed intake during the starter phase was similar in both groups. Starch intake per kg body weight was similar between treatments, but digestible starch intake was slightly lower for the birds in the H group. The amount of starch digested in the first and second half of the small intestine varied considerably between groups. The daily SDS intake per kg body weight was 7 times higher in the H diet than in the L diet.

Performance

Performance of broiler chickens during the experimental period is given in Table 3. During the first two weeks, broiler chickens on the H diet grew faster than those on the L diet ($P < 0.01$) and feed intake was similar for birds in both treatments ($P > 0.05$). This resulted in a lower FCR for broilers in the H group ($P < 0.05$). In the second period (d 15-31), broilers on the H diet consumed more feed ($P < 0.05$) and grew faster ($P < 0.01$) than those on the L diet. FCR was lower for birds on the H diet ($P < 0.01$). In the last period (d 31-38), no effects of site of starch digestion on growth or feed intake were observed ($P > 0.05$). Broilers on the L diet had a lower FCR than those on the H diet ($P < 0.05$). Performance over the whole experimental period showed that chickens on the H diet tended to consume more feed ($P < 0.10$) and grew faster ($P < 0.01$) than those on the L diet. FCR was better for broiler chickens on the H diet compared to those on the L diet (1.73 vs 1.78; $P < 0.01$).

Table 2. Intake parameters of female broiler chickens receiving either high (H) or low (L) amounts of slowly digestible starch (n=6).

Period	Intake parameters (averaged over period)	H	L	SEM
0 – 15 days	Body weight (BW), g	237.0	223.0	3.1
	Feed intake, g/d	38.5	37.9	0.8
	Feed intake, g/d/kg BW	162.2	170.0	2.5
	Starch intake, g/d/kg BW	61.6	61.4	0.9
	Digestible starch intake, g/d/kg BW	56.8	59.8	0.9
	Rapidly digestible starch intake, g/d/kg BW	48.3	58.6	0.8
	Slowly digestible starch intake, g/d/kg BW	8.4	1.2	0.1
15 – 31 days	Body weight, g	897.3	837.2	8.2
	Feed intake, g/d	98.6	95.5	0.9
	Feed intake, g/d/kg BW	109.9	114.1	0.4
	Starch intake, g/d/kg BW	41.8	41.2	0.1
	Digestible starch intake, g/d/kg BW	38.5	40.2	0.1
	Rapidly digestible starch intake, g/d/kg BW	32.7	39.4	0.1
	Slowly digestible starch intake, g/d/kg BW	5.7	0.8	0.0
31 – 38 days	Body weight, g	1591.8	1498.9	11.4
	Feed intake, g/d	134.1	130.1	1.9
	Feed intake, g/d/kg BW	84.2	86.8	1.0
	Starch intake, g/d/kg BW	32.0	31.3	0.4
	Digestible starch intake, g/d/kg BW	29.5	30.5	0.4
	Rapidly digestible starch intake, g/d/kg BW	25.1	29.9	0.3
	Slowly digestible starch intake, g/d/kg BW	4.4	0.6	0.0

Discussion

In this experiment the hypothesis was tested whether the kinetics of starch digestion have an influence on performance of broiler chickens. For this purpose, two diets differing in RDS and SDS content were fed to broiler chickens. Starch containing feedstuffs with known starch digestion coefficients at specific sites of the small intestine of broilers were used (Weurding et al., 2001). In practice, broiler feed is mostly fed as pellets. Pelleting affects starch digestion by particle size reduction and starch gelatinisation. *In vitro* data (not published) show that pelleting increases starch digestion rate of the diet. Furthermore, several authors have shown that *in vivo* starch digestion is improved by pelleting (Carré et al., 1987; Lacassagne at al., 1988; Grosjean et al., 1999). In this experiment, diets were not pelleted because starch digestion coefficients were used which were measured in unpelleted diets. Furthermore, pelleting may reduce differences in RDS and SDS between diets.

Results show that broiler chickens grew faster and more efficient on a diet with a relatively high amount of SDS than on a diet low in SDS. The H diet contained more starch than the L diet. Based on starch digestion coefficients at the posterior ileum from Weurding et al. (2001), the difference in starch content between the diets is expected to be undigested starch. Weurding et al. (2001) concluded that site of starch digestion is not an accurate indicator for starch digestion rate because the mean retention time of digesta in the small intestine is also affected by diet composition. This implies that starch digestion until the end of the jejunum of feedstuffs in this experiment will be slightly different from that in the digestion trial. Mean retention times in the jejunum and ileum varied from 136-182 min in the digestion trial. This difference cannot compensate for the contrast in site of starch digestion in this experiment. On the contrary, mean retention time was inversely correlated to starch digestion coefficients at the different segments of the small intestine. Thus, longer retention times were associated with lower starch digestion coefficients at each site.

Table 3. Weight gain, feed intake and feed conversion ratio of female broiler chickens receiving either high (H) or low (L) amounts of slowly digestible starch (n=6).

Period SDS (g/kg)	Parameter	H 52	L 7	SEM	Starch effect P-value
0 - 15 days	Body weight (g)	434	406	6	< 0.01
	Feed intake (g)	577	568	12	0.62
	FCR	1.464	1.555	0.023	0.02
15 - 31 days	Weight gain (g)	926	862	6	< 0.01
	Feed intake (g)	1578	1528	15	0.04
	FCR	1.703	1.772	0.011	< 0.01
31 - 38 days	Weight gain (g)	463	461	7	0.86
	Feed intake (g)	939	911	13	0.17
	FCR	2.030	1.976	0.016	0.04
0 - 38 days	Body weight (g)	1823	1729	13	< 0.01
	Feed intake (g)	3093	3002	32	0.07
	FCR	1.734	1.777	0.009	0.01

In this experiment, a diet with relatively high amounts of SDS resulted in better performance of broiler chickens than a diet with relatively low amounts of SDS. The data suggest that a certain amount of slowly digestible starch, which is starch that is digested in the lower part of the small intestine, may improve broiler chicken performance. Therefore, starch digestion rate may be a relevant feed characteristic in broiler nutrition. The physiological mechanism(s) responsible for the different responses of broiler chickens to slowly and rapidly digestible starch need to be elucidated. A few possible mechanisms can be suggested. Firstly, rapid glucose absorption results in other metabolic responses than slow glucose absorption. Rapid glucose absorption results in substantial conversions to lactate in the gut wall. This way, the peak glucose supply to the blood is buffered (Riesenfeld et al, 1982). In the liver, lactate is converted back to glucose. When starch digestion is slow, less glucose will be converted to lactate, thus saving energy. Secondly, rate of glucose absorption affects the insulin response after feed intake (Björck et al., 2000) and the insulin sensitivity (Wolever, 2000). Insulin plays a key role in protein deposition during growth (Fox, 1996). A high starch digestion rate results in a rapid, short term increase of the blood insulin level. A slow starch digestion, on the other hand, results in a lower, but longer lasting insulin response. This gradual insulin response may result in a more efficient protein deposition and thus a lower feed conversion ratio for broiler chickens. Thirdly, the level of synchronisation of energy (starch) and protein digestion may affect feed efficiency. These three possible mechanisms are all directly related to starch digestion rate. A fourth possible mechanism is indirectly related to starch digestion rate, but directly related to site of starch digestion. The energy demands of the absorptive- and muscle tissues along the small intestine must be met by nutrients from the diet (McBride and Kelly, 1990; Vaugelade et al., 1994). Glucose and amino acids may be oxidised for this purpose (Fleming et al., 1997). A gradual starch digestion, resulting in a higher glucose supply to the lower part of the small intestine may spare amino acids at that site. Thus, more amino acids may be available for protein deposition (muscle growth) when broiler chickens are fed a diet containing sufficient slowly digestible starch.

The chosen experimental design implied that diet composition, with regard to feedstuffs, differed substantially. Therefore, other than starch related effects cannot be ruled out. These effects could be related to tapioca, sorghum, peas and maize.

Conclusion

Based on the results from this experiment it can be concluded that starch digestion rate may be an important feed characteristic in broiler chickens. Broiler chickens grow faster and more efficient on a diet containing slowly digestible starch. Further research in this area is needed to confirm our findings and to elucidate the mechanisms which are responsible for the better performance of broiler chickens on a diet with slowly digestible starch.

Chapter 6

The Effect of Starch Digestion Rate and Amino Acid Level on Performance of Broiler Chickens

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Abstract

In an experiment with 8160 Cobb broiler chickens, the effect of starch sources with different starch digestion rate on performance was investigated. The hypothesis that starch digestion rate can influence amino acid utilisation was also tested. A second experiment was performed to determine in vivo starch digestion of the most extreme diets. Diets differing in starch digestion rate were established in two ways. Feed formulations were made using native starch sources differing in starch digestion rate. Furthermore, starch sources were processed in different ways to increase starch digestion rate. Feed conversion ratio (FCR) was lower for broilers on diets containing a relatively high amount of slowly digestible starch compared to broilers on diets with rapidly digestible starch. Adding extra amino acids decreased FCR for birds on diets with rapidly digestible starch, but not for birds on diets with slowly digestible starch.
Caecal contents of birds on a diet with slowly digestible starch contained less Clostridium perfringens bacteria than those of birds on diets with rapidly digestible starch. It was concluded that a gradual starch digestion in the small intestine results in better feed efficiency for broiler chickens than a rapid starch digestion. Gradual starch digestion may have an amino acid sparing effect.

Keywords: starch, digestion rate, broiler chickens, amino acids, *Clostridium perfringens*

Introduction

Starch is an important energy source for broiler chickens and it can originate from several different feedstuffs. Starch content and structure in feedstuffs vary considerably. The kinetics of starch digestion in the gastrointestinal tract of broiler chickens is affected by the structure of the starch granules (Oates, 1997) and the botanical structures surrounding the granules (Classen, 1996). Several groups have reported high, but incomplete starch digestion in broiler chickens for cereal grains (Guillaume, 1978; Hesselman and Åman, 1986; Rogel et al., 1987). Some feedstuffs, like legume grains and potatoes, contain a rather high resistant starch fraction for broilers (Yutste et al., 1991; Weurding et al., 2001*a*). Moreover, results from digestion trials by Yutste et al. (1991) and Weurding et al. (2001*a*) indicate that there are major differences in both site and rate of starch digestion. Differences in starch digestion rate for broiler chickens can be predicted from *in vitro* measurements (Weurding et al, 2001*b*). It is of interest to know whether performance is affected by site and / or rate of starch digestion. In a first experiment, it was shown that performance of broiler chickens was better on a diet with slowly digestible starch than on a diet with rapidly digestible starch (Weurding et al., Chapter 5). Differences in starch digestion rate were created using different starch sources and therefore diet composition differed considerably in that experiment. Therefore, an experiment was performed to verify whether the differences in broiler performance were caused by differences in starch digestion rate. In this experiment, two methods were used to obtain variation in starch digestion rate. Starch sources with different rates of starch digestion were selected and for a part of these starch sources starch digestion rate was increased with thermo-mechanical processing.

It was suggested that starch digestion rate may influence metabolic responses like insulin response and / or synchronisation of energy and protein availability (Weurding et al., Chapter 5). It can be hypothesised that slowly digestible starch will lead to a more continuous supply of glucose which may change insulin response (Björck et al., 2000). Insulin plays a key role in protein deposition during growth (Fox, 1996). In addition, this continuous supply of glucose along the gastrointestinal tract enables supply of energy to the gut wall of the posterior part of the small intestine. This may prevent amino acids from being used as an energy source for the gut wall. In order to test this hypothesis, the interaction between starch digestion rate and amino acid content was investigated. Effect of *in vitro* starch digestion rate on ileal starch digestion was studied by an accompanying digestion trial. Diets with slowly digestible starch generally contain more resistant starch than diets with rapidly digestible starch (Björck et al., 2000; Weurding et al., 2001*a*). Resistant starch entering the hind gut can be fermented by microbes in the caeca and this may lead to shifts in the microbial population in the hind gut as was shown for rats (Mallet et al., 1988; Kleessen et al., 1997; Silvi et al., 1999) and pigs (Brown et al., 1997). Therefore the effect of starch digestion rate on bacterial counts in caecal contents was investigated as well.

The objective of this experiment was to study the influence of starch digestion rate on performance of broiler chickens and on the microbial flora in the caeca.

Materials and methods

Animals and housing

Two experiments were performed to investigate the effect of *in vitro* starch digestion rate on ileal starch digestion and performance of broiler chickens.

In experiment 1, 8160 sexed, new-born Cobb 500 male and female broiler chicks, obtained from Cobroed, Lievelde, The Netherlands, were housed in two compartments. Each compartment contained 24 pens and in each pen 85 male and 85 female chicks were housed. Both compartments were divided in three blocks of eight pens. Eight dietary treatments were randomly assigned to a pen in each block.

In experiment 2, 160 new-born, female Cobb 500 broiler chicks (Cobroed, Lievelde) were housed in 16 battery cages (10 chicks per cage) and received a commercial starter diet (supplied by Arkervaart-Twente, Nijkerk, The Netherlands) until they were 14 days old. Temperature decreased gradually from 30 to 24°C. When the chicks were 14 days old, they were assigned to four dietary treatments, based on liveweight.

Treatments were equally distributed across 2 floors in the battery cages.

In both experiments, 23 h light / 1 h dark intervals were used and chicks had unrestricted access to feed and water. Diets were supplied as a mash to maximise the contrasts in starch digestion rate. The experimental protocols of both experiments were in agreement with the standards for animal experiments and were approved by the Ethical Committee of 'De Schothorst'.

Starch sources

Four starch sources were used in the experiments. Selection of starch sources was based on their *in vitro* starch digestion rate: peas (slow), maize and wheat (gradual) and tapioca pellets (rapid). These four starch sources were individually ground with a hammer mill to pass a 2.75 mm screen and were subsequently split in three batches.

Processing

One batch of each starch source received no further treatment, another batch was subjected to steam pelleting and the third batch was subjected to a severe expander pelleting treatment. The steam pelleting treatment involved short time conditioning at approximately 60 °C prior to pelleting through a 6 x 45 mm die. The expander pelleting treatment involved 10 min conditioning at 100 °C prior to expander treatment at 130°C and pelleting through a 4 x 32 mm die. The starch sources were processed in order to increase starch digestion rate to a varying extent. Pellets were crumbled prior to mixing with other dietary ingredients in order to avoid feed intake effects due to structural differences of feedstuffs. However, the expander pelleted maize and tapioca were ground with a hammer mill to pass a 6 mm screen because the pellets were too hard to be crumbled.

Feed formulation

Experiment 1. For each growth stage (starter-, grower- and finisher stage) two diet compositions were formulated. One formulation contained peas and maize as starch sources (PM) and the other formulation contained tapioca and wheat as starch sources (TW). The PM diets contained either untreated peas and maize (PM-0), steam pelleted peas and maize (PM-SP) or expander pelleted peas and maize (PM-EP). Similarly, the TW diets contained either untreated tapioca and wheat (TW-0), steam pelleted tapioca and wheat (TW-SP) or expander pelleted tapioca and wheat (TW-EP). Diets had similar calculated AME and digestible lysine contents and were formulated according to Table 1. Two extra treatments were added in order to test the interaction between starch digestion rate and amino acid content. Diets in these extra treatments were similar to those in treatments PM-0 and PM-EP, but the protein levels were raised by adding 1% casein and 0.5% synthetic glutamine. Therefore, diets in these treatments contained 0.5 g digestible lysine per kg more than the other diets.

Table 1. Composition of experimental diets.

Ingredients (g/kg)	Starter diets			Grower diets			Finisher diets		
	PM[1]	PM+	TW	PM	PM+	TW	PM	PM+	TW
Peas	353.5	348.1	0	352.0	346.7	0	356.0	350.6	0
Maize	353.5	348.1	0	352.0	346.7	0	356.0	350.6	0
Tapioca	0	0	413.0	0	0	425.0	0	0	427.0
Wheat	0	0	160.0	0	0	160.0	0	0	162.0
Casein	0	10.0	0	0	10.0	0	0	10.0	0
L-Glutamine (99%)	0	5.0	0	0	5.0	0	0	5.0	0
Soya beans, extracted	185.5	182.7	268.0	162.0	159.5	228.0	134.5	132.4	263.0
Rape seed, extracted	0	0	0	0	0	0	14.5	14.2	8.9
Potato protein	0	0	30.0	0	0	30.0	0	0	13.0
Feathermeal, hydrolysed	19.5	19.2	20.0	19.3	19.0	20.0	20.0	19.7	20.0
Fish meal	0	0	15.0	0	0	24.5	0	0	0
Soya bean oil	15.7	15.4	19.6	20.0	19.7	26.2	21.3	20.9	28.2
Animal fat	27.5	27.0	42.1	52.6	51.8	60.0	51.2	50.4	52.8
Ground limestone	9.2	9.0	5.0	6.6	6.5	2.0	6.7	6.5	1.6
Monocalcium phosphate	11.6	11.4	11.0	8.3	8.1	7.1	6.9	6.7	7.0
L-Lysine (25%)	1.3	1.2	0.6	0.7	0.6	1.9	0	0	3.1
L-Lysine + L-tryptophane (18% + 5%)	1.2	1.1	0	4.2	4.1	0	5.8	5.7	0
L-Lysine + DL-methionine (20% + 10%)	0	0	0	0	0	0	0	0	2.5
DL-Methionine (10%)	2.7	2.6	0	1.4	3.4	0	3.8	3.7	0
L-Threonine (10%)	0	0	0	0	0	0	7.2	7.0	0
Sodium chloride	1.1	1.0	0.7	0.9	0.8	0.3	0.9	0.8	0.9
Potassium bicarbonate	2.7	2.6	0	2.9	2.8	0	5.2	5.1	0
Vitamin-mineral premix[2]	10.0	9.8	10.0	10.0	9.8	10.0	10.0	9.8	10.0
Anticoccidial premix[3]	5.0	4.9	5.0	5.0	4.9	5.0	0	0	0
Calculated composition (/kg)									
AME (MJ)	11.64	11.64	11.62	12.46	12.45	12.45	12.46	12.44	12.46
Dry matter (g)	876	877	886	880	881	890	880	881	891
Starch[4] (g)	387	381	372	385	379	378	389	383	382
Crude protein (g)	203	214	208	193	204	198	187	199	187
Dig. amino acids (g)	171	182	174	162	173	164	157	168	155
Digestible lysine (g)	10.2	10.7	10.2	10.0	10.5	10.0	9.7	10.2	9.7
Digestible met + cys	7.5	7.6	7.6	7.3	7.5	7.4	7.2	7.4	7.1
Digestible glutamine (g)	30	37	31	29	35	29	27	34	29
Crude fat (g)	66	65	75	94	92	99	94	92	91
Crude fibre (g)	34	33	43	32	32	42	33	33	39
Ash (g)	57	56	69	51	51	62	51	50	56

[1] PM = diets with peas and maize as starch sources, PM+ = diets with peas and maize as starch sources and extra amino acids, TW = diets with tapioca and wheat as starch sources.
[2] This premix contained the following micro-elements (mg/kg): Mn, 7000; Zn, 3700; Fe, 4500; Cu, 1200; I, 100; Se, 15; retinol, 330; cholecalciferol, 5; α-tocopherol, 2500; menadione, 125; thiamin, 50; riboflavin, 500; pyridoxine, 300; cyanocobalamin, 1.5; nicotinic acid, 4000; folic acid, 100; d-pantothenic acid, 800; choline, 20000; biotin, 10; Avilamycin, 1000.
[3] This premix contained (mg/kg): meticlorpindol, 20000; methylbezoquaat, 1670.
[4] Calculated starch content was based on values analysed polarimetrically (Anonymous, 1974).

Experiment 2. Dietary treatments corresponded with treatments PM-0, PM-EP, TW-0 and TW-EP in experiment 1. The corresponding grower diets were diluted with 1.5 g/kg Cr_2O_3 as an indigestible marker.

Sample collection and analysis

Prior to feed formulation, starch content of peas, tapioca, maize and wheat was determined polarimetrically (Anonymous, 1974). Each diet was analysed for contents of dry matter and nitrogen (Dumas). In experiment 1, weight gain and feed intake were recorded in each growth stage (starter: d 0-14; grower: d 14-30 and finisher: d 30-39). Furthermore, feed conversion ratio (g feed intake / g weight gain) and AME conversion ratio (kJ intake / g weight gain) were calculated. AME of diets were corrected for *in vitro* predicted digestible starch content.

Particle size distribution. Particle size distribution (PSD) was determined in grower diets by dry and wet sieve analysis. Differences in particle size distribution determined with dry sieve analysis may affect feed intake. Differences in particle size distribution determined with wet sieve analysis may affect digestibility. For the dry sieve analysis, 100 g material was put on top of a set of 7 sieves: 3.15, 2.5, 2.0, 1.4, 1.0, 0.6, and 0.2 mm. Sieves were vibrated with an amplitude of 2 mm for 4 min (with interruptions) and weight of residues on top of each sieve was determined. For the wet sieve analysis, 50 g material was put in a beaker and 250 ml demineralised water was added. After 60 min soaking, the feed / water mix was stirred for another 60 min at 500 rpm and subsequently put on top of a set of 6 sieves: 2.5, 2.0, 1.4, 1.0, 0.6 and 0.1 mm. Thirty litre of tap water was sprayed through the system, during which the sieves were vibrated with an amplitude of 1 mm (without interruptions). Vibration was stopped 1 min after the tap was closed. Weight of residues on top of each sieve was determined.

***In vitro* starch digestion.** Each grower diet was analysed for starch content (Englyst et al., 1992) and for *in vitro* starch digestion as described by Weurding et al. (2001*b*). In this procedure, which simulates the consecutive digestive processes in the various parts of the broiler alimentary tract, test tubes containing the feed sample, glass balls, a mixture of digestive enzymes and a buffer solution were incubated in a shaking water bath (37°C). After each of nine incubation times (0.25, 0.50, 0.75, 1, 2, 3, 4, 5 and 6 h), aliquots were taken from the tubes and the amount of released glucose was measured colorimetrically. Starch digestion coefficients (*DC*) were calculated for each incubation time. Rapidly digestible starch (RDS_p), slowly digestible starch (SDS_p) and resistant starch (RS_p) fractions for poultry were calculated from the *DC* as measured after 2 and 4 h (DC_2 and DC_4). *In vitro* starch digestion after 2 and 4 h correlated best with *in vivo* starch digestion until the posterior jejunum and the posterior ileum of 4 week old broiler chickens respectively (Weurding et al., 2001*b*). RDS_p (%) was defined as $1.16 \cdot DC_2 - 21.5$, SDS_p (%) as $1.29 \cdot DC_4 - 30.9 - RDS_p$ and RS_p (%) as $100 - RDS_p - SDS_p$. The starch digestion coefficient (*DC*) was plotted against time (*t*) to give an exponential rate curve ($DC_t = D \cdot (1 - e^{-k(d) \cdot t})$ from which the potentially digestible starch fraction (*D*, %) and the starch digestion rate (k_d, /h) were estimated. The *D* fraction represents the asymptote of the digestion curve and the k_d determines the steepness of the curve (a higher k_d means that the curve is steeper).

Determination of microbial counts. At d 36 of experiment 1, one hen was removed from each pen from treatments PM-0, PM-EP and TW-0. These 18 birds were euthanised by an intravenous injection of T61, this is an aqueous solution containing 200 g embutramide, 50 g mebezoniumiodide and 5 g tetracainehydrochloride per litre (Hoechst Veterinär GmbH, München, Germany). Immediately after injection, caeca were removed from the dead bird, caeca contents were gently squeezed out and prepared for counts of *Clostridium perfringens* (NCFA, 1997) and *Lactobacilli* (Smits et al., 1998).

***In vivo* starch digestion.** In experiment 2, the birds were euthanised by an intravenous injection of T61 on d 28. Immediately after injection, the small intestine was removed from the dead animal. Jejunum and ileum were separated at Meckel's diverticulum. Both jejunum and ileum were split in two parts of equal length: anterior jejunum (AJ), posterior jejunum (PJ), anterior ileum (AI) and posterior ileum (PI) respectively. Digesta were rinsed out of each segment (without squeezing) with demineralised water (4°C) into separate aluminium containers. Digesta were stored at –20°C and subsequently freeze-dried. After freeze-drying, the samples were pre-ground with a pestle and mortar and subsequently ground in a Retsch mill to pass a 1 mm screen. Starch and Cr_2O_3 were determined in experimental diets and freeze-dried digesta from the PJ, AI and PI. Starch was analysed according to Englyst et al. (1992). Cr_2O_3-content was determined by wet destruction with a mixture of $HNO_3/HClO_4$ (1:1). The absorption of the hexavalent Cr atom, measured at a wavelength of 357.8 nm, is proportional to the Cr_2O_3-concentration in the sample.

Statistical analysis

For experiment 1, effects of starch sources and processing on body weight, weight gain, feed intake, feed conversion ratio and AME conversion ratio were tested using a model that included block, starch source, processing and the interaction between starch source and processing. The effect of adding extra amino acids to diets with untreated and expanded peas and maize was tested using a model that included block, processing, amino acid level and the interaction between processing and amino acid level. Analysis of variance was performed using the GLM procedure of SAS (SAS Institute, 1989). Means were separated by calculating the least significant difference.

For experiment 2, starch digestion coefficients were transformed by the logit transformation {ln [p/(100-p)]} prior to statistical analysis in order to meet statistical assumptions (normal distribution and homogeneity of variance). Effects of starch sources and processing on starch digestion were tested using a model that included floor, replication, starch source, processing and the interaction between starch source and processing.

Results

Particle size distribution

Particle size distributions (PSD), as determined in the grower diets by dry and wet sieve analysis are presented in Figure 1. Dry sieve analysis showed that diets with steam pelleted starch sources had more coarse particles than diets with untreated starch sources. PSD of diets with expander pelleted starch sources were in between. Differences in dry sieve analysis between PM and TW diets, within processing treatments, were not observed. Mean particle size was 0.76 and 0.74 mm for PM-0 and TW-0, 1.34 and 1.41 mm for PM-SP and TW-SP and 0.90 and 1.00 mm for PM-EP and TW-EP respectively. Wet sieve analysis showed that PM-0 and PM-SP diets were slightly coarser than the PM-EP diet. The latter diet had a similar PSD as the three TW diets, where no differences between treatments were observed. Mean particle size was 0.56, 0.47 and 0.40 mm for PM-0, PM-SP and PM-EP respectively. All three TW diets had a mean particle size of 0.40 mm.

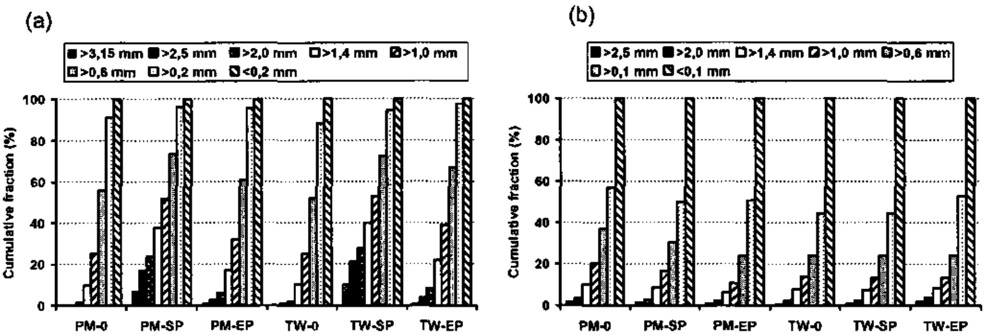

Figure 1. Cumulative particle size distribution of grower diets as determined with dry (a) and wet (b) sieve analysis.

In vitro starch digestion

The estimated potentially digestible starch fraction (D) was 100% for TW diets and close to 100% for PM diets (Table 2). Starch digestion rate (k_d) of PM-0 was lower than that of TW-0. Steam pelleting increased starch digestion rate and expander pelleting increased starch digestion rate even more. Processing effects on starch digestion rate were most pronounced for the PM formulation. Difference in starch digestion rate between PM-0 and TW-0 was similar to that between PM-0 and PM-EP. This is also reflected in the predicted rapidly digestible starch (RDS_p) fractions. Predicted starch digestion (DS_p) was incomplete for 4 out of 6 diets. Diets from treatments PM-0 and PM-SP showed a particularly poor starch digestion compared to the TW diets. Processing had a pronounced effect on the slowly digestible starch

(SDS_p) fraction of PM diets. Expander pelleting reduced SDS_p from 215 to 62 g/kg starch. SDS_p fraction in TW diets was hardly affected by processing and was at a similar level as that in PM-EP (62 g/kg starch).

Table 2. Starch characteristics of grower diets[1].

Diet	Starch[2] (g/kg)	RDS_p (g/kg starch)	SDS_p (g/kg starch)	RS_p (g/kg starch)	DS_p (g/kg starch)	*D* (g/kg starch)	k_d (/h)
PM-0[3]	359	707	215	78	922	998	0.84
PM-SP	362	777	166	57	943	969	1.26
PM-EP	372	913	62	26	974	987	2.39
TW-0	337	917	66	17	983	1000	1.98
TW-SP	336	939	62	0	1000	1000	2.45
TW-EP	334	966	59	–26	1026	1000	2.95

[1] Potential starch digestibility (*D*) and fractional starch digestion rate (k_d) were estimated from *in vitro* starch digestion coefficients at different incubation times according to $DC_t = D * (1 - e^{-k(d) \cdot t})$. Rapidly digestible starch (RDS_p), slowly digestible starch (SDS_p), resistant starch (RS_p) and digestible starch (DS_p) were calculated as explained in the text.
[2] Starch was analysed enzymatically according to Englyst et al. (1992).
[3] PM = peas and maize based diet; TW = tapioca and wheat based diet; 0 = starch sources untreated; SP = starch sources steam pelleted; EP = starch sources expander pelleted.

Feed and starch intake

In Table 3, intake parameters are presented. In the starter phase, relative feed intake (g / kg bodyweight) was higher, but relative starch intake was lower for birds on TW diets. In the grower phase, relative feed intake was similar for PM and TW diets, but relative starch intake was lower for TW diets. In the finisher phase, relative feed intake was higher for birds on TW diets, but relative starch intake was similar for PM and TW diets. Relative intake of slowly digestible starch was 3.5 (PM-0) to 2 (PM-SP) times higher in diets with slowly digestible starch compared to diets with rapidly digestible starch (PM-EP and TW diets).

Performance

Performance data from experiment 1 are presented in Tables 4 and 5.

Starch sources and processing. Feed intake from d 0-39 was significantly increased by processing of tapioca and wheat. Differences were smaller for diets with peas and maize and therefore an interaction was found between starch source and processing. Processing affected weight gain ($P < 0.01$), particularly for birds on TW diets. Weight gain was not affected by starch source within processing treatments. Birds on the PM-0 diet tended to be heavier than those on the TW-0 diet ($P = 0.11$). Feed conversion ratio (FCR) was lower for birds on PM diets within and across ($P < 0.01$) processing treatments. There was a tendency to an interaction between starch source and processing on FCR. Steam pelleting improved FCR for birds on PM diets, but did not affect FCR for birds on TW diets ($P = 0.11$). An interaction on AME conversion ratio (AMECR) was also observed between processing and starch source ($P < 0.01$). Expander pelleting increased AMECR for birds on the PM diets from 20.8

to 21.7, but AMECR for birds on the TW diets was unaffected by processing (21.4 to 21.7). AMECR was best for birds on the PM-0 and PM-SP diets.

Table 3. Intake parameters (g/d/kg bodyweight unless specified otherwise) of broiler chickens on experimental diets.

Period (days)	Intake parameters[1]	PM[2]			TW			SEM
		0	SP	EP	0	SP	EP	
d 0-14	Body weight, g	225.8	243.0	233.3	224.4	234.8	233.4	2.3
	Feed intake, g/d	35.3	37.5	35.9	34.9	37.5	37.1	0.4
	Feed intake	156.1	154.4	153.7	155.6	159.7	158.8	0.7
	Starch intake	56.0	55.9	57.2	52.4	53.7	53.0	0.2
	Digestible starch intake	51.7	52.7	55.6	51.5	53.7	53.0	0.2
	Rapidly digestible starch intake	39.7	43.4	52.3	48.1	50.5	51.3	0.2
	Slowly digestible starch intake	12.0	9.3	3.4	3.4	3.2	1.7	0.0
d 14-30	Body weight, g	924.5	963.7	930.9	900.4	948.5	937.6	8.1
	Feed intake, g/d	107.4	109.0	108.3	104.6	112.3	108.1	1.4
	Feed intake	116.2	113.1	116.3	116.2	118.5	115.3	1.4
	Starch intake	41.7	40.9	43.3	39.2	39.8	38.5	0.5
	Digestible starch intake	38.4	38.6	42.1	38.5	39.8	38.5	0.5
	Rapidly digestible starch intake	29.5	31.8	39.5	35.9	37.4	37.2	0.4
	Slowly digestible starch intake	8.9	6.8	2.6	2.6	2.4	1.3	0.1
d 30-39	Body weight, g	1762.0	1811.8	1763.7	1715.4	1809.1	1773.0	16.1
	Feed intake, g/d	156.1	152.9	154.1	163.2	170.4	167.1	2.2
	Feed intake	88.6	84.4	87.4	95.2	94.2	94.3	0.9
	Starch intake	31.8	30.5	32.5	32.1	31.6	31.5	0.3
	Digestible starch intake	29.3	28.8	31.6	31.5	31.6	31.5	0.3
	Rapidly digestible starch intake	22.5	23.7	29.7	29.4	29.8	30.4	0.3
	Slowly digestible starch intake	6.8	5.1	1.9	2.1	1.9	1.0	0.0

[1] Intake parameters are averaged over the period. Digestion related parameters were based on *in vitro* measurements.

[2] PM = peas and maize based diet; TW = tapioca and wheat based diet; 0 = starch sources untreated; SP = starch sources steam pelleted; EP = starch sources expander pelleted, $n = 6$.

In the starter phase, processing affected feed intake and body weight ($P < 0.01$). Expander pelleting and particularly steam pelleting of starch sources resulted in a higher feed intake and body weight of birds compared to untreated starch sources. An interaction between starch source and processing was observed for FCR and AMECR. Both processing methods resulted in a lower FCR for birds on PM diets, whereas they increased FCR for birds on TW diets ($P < 0.01$). Expander pelleting increased AMECR for birds on PM diets from 14.7 to 15.1. For TW diets, both steam pelleting and expander pelleting increased AMECR from 14.7 to 15.0 and 14.9 respectively. Performance was not affected by starch source when untreated. However, bodyweight ($P < 0.05$), FCR and AMECR ($P < 0.01$) were better for birds on the PM-SP diet than for birds on the TW-SP diet. Birds on the PM-EP diet consumed less than ($P < 0.05$), but had the same bodyweight as birds on the TW-EP diet. This resulted in a better FCR for birds on PM-EP compared to birds on TW-EP ($P < 0.01$). AMECR was unaffected by starch source when they were expander pelleted ($P > 0.05$).

Table 4. The effects of starch source (SS) and processing (PROC) on broiler performance.

Growth stage	SS	PM[1]			TW				P-values		
	PROC	0	SP	EP	0	SP	EP	SED	SS	PROC	SS x PROC
d 0-14	Body weight, g	412^{c}	446^{a}	427^{b}	409^{c}	430^{b}	427^{b}	7	0.12	< 0.01	0.20
	Feed intake, g	494^{b}	525^{a}	502^{b}	489^{b}	525^{a}	519^{a}	8	0.40	< 0.01	0.13
	FCR, g/g	1.329^{bc}	1.294^{d}	1.300^{d}	1.326^{c}	1.348^{a}	1.343^{ab}	0.008	< 0.01	0.45	< 0.01
	AMECR, kJ/g	14.70^{b}	14.55^{b}	15.08^{a}	14.67^{b}	15.03^{a}	14.92^{a}	0.09	0.06	0.01	< 0.01
d 14-30	Weight gain, g	1026^{a}	1035^{a}	1009^{ab}	983^{b}	1038^{a}	1021^{a}	17	0.35	0.03	0.06
	Feed intake, g	1718^{bc}	1744^{ab}	1733^{bc}	1674^{c}	1797^{a}	1729^{bc}	31	0.92	< 0.01	0.10
	FCR, g/g	1.675	1.686	1.718	1.703	1.733	1.693	0.026	0.28	0.51	0.14
	AMECR, kJ/g	19.94^{c}	20.36^{bc}	21.39^{a}	20.28^{bc}	20.78^{ab}	20.24^{bc}	0.31	0.48	0.01	< 0.01
d 30-39	Weight gain, g	650	661	657	647	684	650	22	0.76	0.30	0.60
	Feed intake, g	1405^{c}	1376^{c}	1387^{c}	1469^{b}	1534^{a}	1504^{ab}	26	< 0.01	0.64	0.06
	FCR, g/g	2.165^{bc}	2.085^{c}	2.125^{c}	2.272^{a}	2.249^{ab}	2.318^{a}	0.049	< 0.01	0.23	0.46
	AMECR, kJ/g	25.76^{cd}	25.19^{d}	26.44^{bc}	27.05^{ab}	26.96^{abc}	27.70^{a}	0.59	< 0.01	0.07	0.79
d 0-39	Body weight. g	2087^{bc}	2143^{ab}	2092^{abc}	2039^{c}	2151^{a}	2098^{abc}	29	0.50	< 0.01	0.32
	Feed intake, g	3613^{c}	3640^{c}	3611^{c}	3627^{c}	3846^{a}	3743^{b}	50	< 0.01	< 0.01	0.04
	FCR, g/g	1.765^{b}	1.731^{c}	1.760^{b}	1.815^{a}	1.822^{a}	1.819^{a}	0.014	< 0.01	0.30	0.11
	AMECR, kJ/g	20.82^{b}	20.73^{b}	21.74^{a}	21.42^{a}	21.67^{a}	21.56^{a}	0.17	< 0.01	< 0.01	< 0.01

[1] PM = peas and maize based diet; TW = tapioca and wheat based diet; 0 = starch sources untreated;
SP = starch sources steam pelleted; EP = starch sources expander pelleted.

Means in a row without a common superscript differ, P< 0.05.

n = 6.

In the grower phase, processing effects on feed intake ($P < 0.01$) and weight gain ($P < 0.05$) were similar as in the starter phase. FCR was not affected significantly. AMECR was higher for PM-EP compared to PM-0 and PM-SP. Weight gain was better for birds consuming the PM-0 diet than those consuming the TW-0 diet ($P < 0.05$). Birds on TW-SP tended to consume more feed than those on PM-SP ($P < 0.10$), but weight gain was unaffected. AMECR was better for birds on the TW-EP diet than for those on the PM-EP diet.

In the finisher phase, feed intake, FCR and AMECR were affected by starch source ($P < 0.01$). Feed intake and FCR of birds on PM diets were 1389 g and 2.13 compared to 1502 g and 2.28 for birds on TW diets. The interaction between starch source and processing on feed intake was almost significant ($P < 0.10$). Birds on PM diets consumed the least and birds on TW diets consumed most when starch sources were steam pelleted. Weight gain was not affected by treatments.

Amino acid level. Amino acid level did not affect feed intake and weight gain in any period. In the starter phase, a tendency to an interaction between processing and amino acids was observed for FCR ($P = 0.07$) and AMECR ($P = 0.09$). FCR and AMECR of birds on the PM-0 diet improved with extra amino acids (Table 5), but FCR and AMECR of birds on the PM-EP diet was not affected by amino acid level.

Table 5. Effect of extra amino acids (+ AA) in diets with untreated (0) or expander pelleted EP) peas and maize (PM) on bird performance.

Growth stage		PM-0		PM-EP		SEM	P-value		
		0	+ AA	0	+ AA		PROC	AA	PROC x AA
d 0-14	Body weight, g	412	419	427	420	4	0.09	0.91	0.14
	Feed intake, g	494	497	502	496	6	0.56	0.79	0.45
	FCR, g/g	1.329	1.311	1.300	1.305	0.006	< 0.01	0.27	0.07
	AMECR, kJ/g	14.70	14.51	15.08	15.13	0.07	< 0.01	0.29	0.09
d 14-30	Weight gain, g	1026	1026	1009	1035	11	0.71	0.27	0.28
	Feed intake, g	1718	1707	1733	1693	23	0.99	0.29	0.53
	FCR, g/g	1.675	1.665	1.718	1.638	0.013	0.56	< 0.01	0.02
	AMECR, kJ/g	19.94	19.81	21.39	20.38	0.17	< 0.01	< 0.01	0.02
d 30-39	Weight gain, g	650	658	657	659	13	0.75	0.71	0.80
	Feed intake, g	1405	1432	1387	1383	25	0.19	0.65	0.55
	FCR, g/g	2.165	2.180	2.125	2.099	0.032	0.08	0.86	0.54
	AMECR, kJ/g	25.76	25.94	26.44	26.12	0.40	0.30	0.86	0.55
d 0-39	Body weight, g	2087	2103	2092	2114	19	0.66	0.33	0.88
	Feed intake, g	3613	3633	3611	3564	38	0.37	0.73	0.39
	FCR, g/g	1.765	1.761	1.760	1.719	0.011	0.06	0.07	0.11
	AMECR, kJ/g	20.82	20.78	21.74	21.22	0.14	<0.01	0.06	0.10

$n = 6$.

In the grower phase, another interaction was observed ($P = 0.02$). FCR and AMECR of birds on the PM-0 diet was not affected by extra amino acids, but FCR and AMECR of birds on the PM-EP diet improved from 1.72 to 1.64 and from 21.39 to 20.38 kJ intake / g weight gain with extra amino acids. The interaction was less pronounced when measured over the whole growth period ($P = 0.11$ and 0.10 for FCR and AMECR respectively).

Effects on microbial counts

Results on bacteria colony forming units (cfu) are shown in Figure 2. Broiler chickens receiving the PM-0 diet contained respectively 100 and 32 times less cfu of Clostridia perfringens than broiler chickens receiving the PM-EP and TW-0 diets. No differences in *Lactobacilli* cfu were observed.

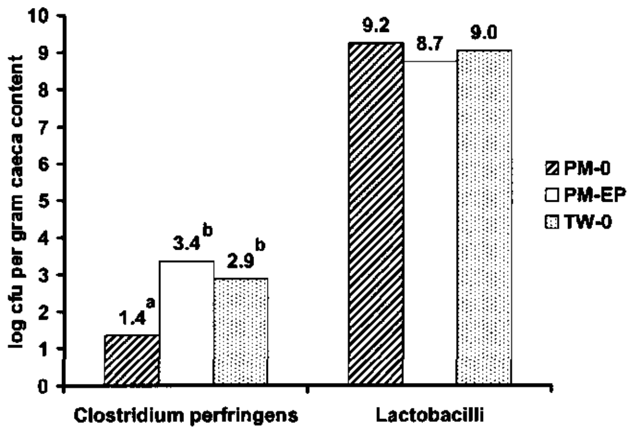

Figure 2. Effect of diet formulation and processing on bacterial counts in the caeca of broiler chickens (cfu = colony forming units). Means for bars with different superscript differ significantly ($P<0.05$). $n = 6$.

In vivo starch digestion

At the posterior jejunum and both the anterior and posterior ileum, starch digestion was less for the PM-0 diet than that for the PM-EP, TW-0 and TW-EP diets (Table 6). Starch digestion of the PM-EP and TW-0 diets was similar. Nine percent of dietary starch remained undigested in the PM-0 diet. Expander pelleting decreased this fraction to 3.4%. Only 1.9 and 0.7% of starch in the TW-0 and TW-EP diets respectively remained undigested at the end of the small intestine. *In vivo* starch digestion coefficients at the posterior ileum corresponded well with predicted starch digestion coefficients (DC_p) based on *in vitro* measurements (Table 2). *In vivo* starch digestion coefficients at the posterior jejunum were underestimated by the *in vitro* method (RDS_p). Using tapioca and wheat instead of peas and maize increased starch digestion coefficients in each gut segment ($P < 0.0001$). Expander pelleting increased starch digestion coefficients in both PM and TW diets ($P < 0.0001$). An interaction between starch sources and processing was found for starch digestion at the posterior jejunum. In this gut segment, the effect of expander pelleting was more pronounced on the PM diet than on the TW diet ($P < 0.01$).

Table 6. Effect of starch sources and expander pelleting on *in vivo* starch digestion coefficients in three segments of the small intestine of broiler chickens.

Starch sources	Starch digestion coefficient (SD)[1]		
	Posterior jejunum	Anterior ileum	Posterior ileum
		%	
PM-0	79.8 (1.4)	89.3 (1.4)	91.2 (0.6)
PM-EP	94.7 (0.2)	96.3 (0.3)	96.6 (0.5)
TW-0	95.8 (0.6)	97.5 (0.4)	98.1 (0.4)
TW-EP	98.6 (0.0)	99.2 (0.1)	99.3 (0.1)
P-values	< 0.01	< 0.01	< 0.01
	< 0.01	< 0.01	< 0.01
	< 0.01	0.80	0.62

[1] SD = standar deviation; PM = peas and maize based diet; TW = tapioca and wheat based diet; 0 = starch sources untreated; EP = starch sources expander pelleted.
$n = 4$.

Discussion

In these experiments, contrasts in starch digestion rate were established in two ways. Firstly by using a rapidly (tapioca) versus a slowly digestible starch source (peas). Secondly by using untreated versus heat treated starch sources. Thermo-mechanical processes are known to gelatinise starch to a varying extent (Thomas et al., 1999; Goelema et al., 1999). Due to the different heat treatments (steam pelleting and expander pelleting), differences in starch digestion rate were introduced within starch sources. Differences in feed intake can be related to differences in particle size as determined by dry sieve analysis. More coarse particles generally results in a higher feed intake by broiler chickens (Nir et al., 1990; Nir et al., 1994). Diets with steam pelleted and subsequently crumbled starch sources had more coarse particles than diets with expander treated and subsequently ground starch sources. Diets with untreated starch sources had the least coarse particles. The differences in feed intake in this experiment are in line with differences in particle size distribution as determined with dry sieve analysis. Particle size distribution (PSD) as determined with wet sieve analysis reflects the PSD of the feed particles after the feed has dissolved in the gut of the chicken. PSD of the feed particles in the gut may affect their digestibility. Differences in particle size as determined with wet sieve analysis were small, indicating that differences in digestibility due to particle size are not likely. The apparent metabolisable energy (AME) content of feedstuffs is affected by digested components (Fisher, 2000; Centraal Veevoederbureau, 1999). Wiseman et al. (2000) and Rogel et al. (1987) observed a positive correlation between AME and starch digestibility in wheat. Undoubtedly, AME content of other starch sources is also affected by starch digestibility. From Table 2 it can be seen that the TW grower diets had a substantially lower starch content than the PM grower diets. The polarimetric method, which was used for starch analysis of starch sources prior to feed optimisation for this experiment, obviously resulted in higher starch contents than the enzymatic method. The starch fraction measured with the polarimetric method may contain non starch components, which may be variable between feedstuffs. Differences in starch content between TW and PM diets are similar in starter and finisher diets, because the same batches were used in these feeds. Therefore, the AME content in the TW diets is likely to be lower than in the PM diets. This may explain the observation that the birds on the PM-EP diet showed a substantially better FCR than the birds on the three TW diets (1.76 vs. 1.82 over the whole experimental period), although predicted SDS_p contents were similar (62 vs. 66, 62 and 59 g SDS / kg starch).

Based on earlier findings (Weurding et al, Chapter 5), it was expected that birds on the PM-0 diet (slowest starch digestion) would show the best FCR. However, FCR for birds on this diet was similar to that of birds on the PM-EP diet (1.76), which is higher than that of birds on the PM-SP diet (1.73). Both *in vitro* and *in vivo* measurements showed a lower (predicted) ileal starch digestion for the diet with untreated peas and maize compared to that of diets with rapidly digestible starch. Therefore, AME conversion ratios were calculated as AME intake (kJ) per g weight gain. Differences in enzymatically analysed starch content and predicted ileal starch digestion coefficients were taken into account in this calculation. AMECR was 20.8, 20.7 and 21.7 for PM-0, PM-SP and PM-EP diets respectively. For TW-0, TW-SP and TW-EP these values were 21.4, 21.7 and 21.6 respectively. Birds receiving diets PM-0 and PM-SP received relatively high amounts of slowly digestible starch and had the most efficient AMECR ($P < 0.01$). Severe heat treatments may enhance the Maillard reaction in which lysine is destroyed and / or bound to sugars rendering it unavailable. The lysine content in diets PM-0 and PM-EP was 11.83 and 11.86 respectively. This indicates that heat damage due to the expander treatment is not likely.

These results confirm earlier findings in which a diet with slowly digestible starch resulted in a better FCR of broiler chickens compared to a diet with rapidly digestible starch (Weurding et al., Chapter 5). Using different starch sources and using processing techniques to create differences in starch digestion rate resulted in similar contrasts in FCR. However, to our knowledge, other groups have never shown results that fit this picture. For wheat specifically, positive relations between starch digestion rate and weight gain (Waldron et al., 1995) and between starch digestion rate and AME content (Wiseman et al., 2000) were reported. This is in contrast with our results. The contrasts in starch digestion rate, however were much bigger in our experiments, because we used different starch sources and severe processing techniques. Furthermore, differences in starch digestion rate between wheat varieties may be related to variation in soluble NSP and its effect on digesta viscosity. After our first growth trial we postulated four possible mechanisms for improved FCR when a diet with slowly digestible starch is fed to broiler chickens (Weurding et al., Chapter 5). The fact that extra amino acids improved FCR and AMECR of broilers on a diet with rapidly digestible starch, but not that of broilers on a diet with slowly digestible starch suggests a protein sparing effect of slowly digestible starch.

The positive effect of slowly digestible starch is accomplished in the period from d 14-39 (grower and finisher phase). In the starter phase, another interaction between starch source and processing was observed. Diets with processed peas and maize resulted in better FCR and AMECR than the diet with untreated peas and maize. On the other hand, diets with processed tapioca an wheat resulted in a higher FCR and AMECR than the diet with untreated tapioca and wheat. Production is normally measured over six weeks, but starch digestion was measured at a specific point of time during this production period (after 4 weeks). It is well known that digestion is not constant in young animals. In the starter phase (d 0-14), when chicks are still young, starch digestion capacity is still increasing (Uni et al., 1995). Nitsan et al. (1991) showed that maximal amylase activity in the pancreas was reached after 8 days and maximal amylase activity in the small intestine was reached after 17 days of age. Lipase, trypsin and chymotrypsin activities were also lower in younger birds. A lower starch digestion capacity in young birds implicates a slower starch digestion. When starch digestion is already slow, but complete in a four week old bird, than a lower starch digestion rate in the young animal may result in an incomplete starch digestion. Therefore, the contrasts in digestible starch intake (Table 3) were probably bigger during the starter phase and this may explain why FCR and AMECR in the starter phase (d 0-14) were better for birds on diets with processed peas and maize than for birds on diets with untreated peas and maize. In the case of untreated tapioca and wheat, starch digestion at 4 weeks was rapid. The lower starch digestion capacity in the young birds may result in a slower, but still complete starch digestion in the untreated tapioca and wheat. Diets with processed tapioca and wheat were probably rapidly digestible even by young birds and this may explain why processing of these feedstuffs impairs energy efficiency (effect of slowly digestible starch). If energy (starch) was limiting weight gain of birds on untreated peas and maize in the starter phase, then the extra glucogenic amino acids may have been used as an energy source. This can explain why FCR and AMECR of birds on the starter diet with untreated peas and maize improved with extra amino acids and FCR and AMECR of birds on the diet with expander treated peas and maize did not.

Several authors observed shifts in microbial flora after feeding resistant starch to rats (Mallet et al., 1988; Kleessen et al., 1997; Silvi et al., 1999) or pigs (Brown et al., 1997). Increased resistant starch flux through the lower tract reduced *Clostridium perfringens* cfu in the caeca in our experiment. This is a positive effect, because *Clostridium perfringens* is known to cause necrotic enteritis.

The results of these experiments confirm the hypothesis that the kinetics of starch digestion are relevant for broiler nutrition. Starch digestion rate affects growth efficiency of broiler chickens and is related to protein utilisation. Feeding a diet containing gradually digestible starch results in better feed efficiency and lower counts of *Clostridium perfringens* bacteria.

Chapter 7

The Relation Between Starch Digestion Rate and Amino Acid Level in Broiler Chicken Nutrition

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Abstract

Digestion coefficients of nutrients give information about the amount of nutrients available to the animal, but not about the rate or site of absorption. A gradual starch digestion may have an amino acid sparing effect and therefore enhance growth efficiency of broiler chickens. A growth trial was performed with 6800 broiler chickens from 9-30 d of age to investigate interaction between starch digestion rate and amino acid level. Birds were fed either a pea/maize based diet (slowly digestible starch) or a tapioca/maize based diet (rapidly digestible starch). Both type of diets were formulated with five levels of digestible lysine, varying from 8.5 to 11.0 g/kg. The minimal levels of other amino acids varied accordingly. Starch source did not affect feed intake (2213 g), but weight gain was consistently higher for birds on pea/maize diets than for those on tapioca/maize diets (1426 vs 1400 g; $P < 0.01$). Feed conversion ratio was better ($P < 0.01$) for birds on pea/maize diets (1.55) than for birds on tapioca/maize diets (1.58). The difference in feed conversion ratio between birds on pea/maize- and tapioca/maize diets was bigger with lower amino acid levels (0.043) than with higher amino acid levels (0.019) in the diet ($P = 0.11$). This interaction was more pronounced during the first nine days of the experiment ($P < 0.05$). It was concluded that feeding slowly digestible starch improves protein- and energy utilization in broiler chickens.

Keywords: starch digestion rate, amino acid level, broiler chickens, peas, tapioca

Introduction

Feed evaluation in monogastric animals is based on digested nutrients. Digestion coefficients of nutrients at the terminal ileum give information about the amount of nutrients available to the animal, but not about the site nor the synchronization of availability of different nutrients. The major part of starch is digested in the upper part of the small intestine. Diets with rapidly digestible starch may result in elevated plasma glucose levels at times when other nutrients are not yet absorbed. This may have consequences for protein utilization. Diets with similar amounts of digestible nutrients, but differences in digestion kinetics may result in different performance. In ruminant nutrition, the importance of the kinetics of carbohydrate and protein digestion in the rumen has long been recognized. In human nutrition, the glycaemic index of foodstuffs is used to manipulate glucose absorption rate in order to prevent metabolic disorders or to enhance athlete performance (Brand-Miller, 1999). Weurding et al. (Chapter 6) observed differences in performance of broiler chickens receiving diets that were iso-energetic, but different in terms of starch digestion rate. Feeding diets containing slowly digestible starch resulted in a better feed conversion for broiler chickens than feeding diets with rapidly digestible starch. Moreover, an interaction between starch digestion rate and amino acid content was observed. Adding casein and glutamine to a diet containing slowly digestible starch (SDS) did not improve feed conversion ratio (FCR), but when casein and glutamine were added to a diet containing rapidly digestible starch, FCR improved. These results may be related to the efficiency of protein deposition which is affected by insulin levels. The energy supply to the intestinal wall may also be involved. Vaugelade et al. (1994) stated that intestinal transport of absorbed nutrients coincides with their partial catabolism in the gut. The gastrointestinal tract consumes approximately 20% of all dietary energy to support digestive and absorptive processes. Therefore, metabolic activity of the small intestine also affects the supply of nutrients to other tissues in the body (Cant et al., 1996). Glutamine and glucose are preferentially used to provide energy for the small intestine (Fleming et al.,1991; Fleming et al., 1997), but other amino acids have also been mentioned as energy sources for gut tissues. Diets in which all starch is digested in the upper small intestine will not provide the lower part with glucose for its energy demands. In that case, more amino acids will be oxidized for that purpose. Diets containing starch which is partly digested in the lower small intestine (slowly digestible starch), supply that part with glucose, thereby sparing amino acids from being oxidized. Our hypothesis is that the effect of starch digestion rate on performance is more pronounced with lower amino acid levels in the diet. The objective of this experiment is to investigate whether the effect of amino acid supply on performance of broiler chickens depends on the kinetics of starch digestion.

Materials and methods

Animals and Housing

An experiment was performed with 6800 sexed one-day-old male and female broiler chickens of the Cobb 500 strain. Chicks were obtained from Cobroed, Lievelde, The Netherlands and were housed in two compartments. For this experiment, 20 pens per compartment were used and in each pen 85 male and 85 female chicks were housed. Both compartments were divided in two blocks of 10 pens each. From d 0-9, chicks were fed a starter diet containing peas, tapioca and maize. From d 9-30, chicks were fed one of ten experimental diets, which were randomly assigned to a pen in each block. A 23 h light and 1 h dark interval was used and chicks had unrestricted access to feed and water. The experimental protocol was in agreement with the standards for animal experiments and were approved by the Ethical Committee of 'De Schothorst'. Average body weight at the start of the experimental period (d 9) was 230 g. This experiment was part of a larger experiment in which the response of birds to varying levels of slowly digestible starch was investigated (Weurding et al., Chapter 8).

Diets

Three starch sources were selected for the experiment. The selection was based on *in vitro* starch digestion rate: peas (slow), maize (gradual) and tapioca pellets (rapid).

Two grower diets containing peas and maize (PM) and two grower diets containing tapioca and maize (TM) were formulated with digestible lysine contents of 8.50 (PM1 and TM1) and 11.00 g/kg (PM5 and TM5) respectively. In order to get diets with intermediate digestible lysine contents, diets with the same starch sources were mixed to give digestible lysine contents of 9.13, 9.75 and 10.38 g/kg (PM2, PM3, PM4, TM2, TM3 and TM4 respectively). During formulation of the diets, the minimal ratio of other digestible amino acids to digestible lysine was the same in all diets. Diets were conditioned with steam at 55-58°C for ±10 sec and subsequently pelleted through a 3 x 80 mm die (exit temperature of pellets varied from 58-62°C). Composition of diets is given in Table 1.

Analysis

Each experimental diet was analysed for dry matter, nitrogen (Dumas) and starch (Brunt et al., 1998). Two diets with 9.75 g digestible lysine per kg (PM3 and TM3) were additionally analysed for particle size distribution, pellet quality, starch (Englyst et al., 1992) and *in vitro* starch digestion (Weurding et al., 2001*b*).

Pellet Quality. Pellet quality (Table 2) was measured by means of durability, hardness and percentage of fines (Payne et al., 1994). Percentage of fines is determined by means of screening of the pellets. The mesh width should be slightly less than the pellet diameter. Durability is the most important aspect of pellet quality and means the ability of pellets to withstand the stresses of handling and delivery without breaking up. It was measured with the tumbling can method, known as Pfost. In this method 500 g of screened pellets were tumbled for 10 min at 50 rpm. The sample was then screened again and the whole pellets were weighed. The percentage of whole pellets remaining is expressed as the durability. Hardness is important to avoid breakdown due to pressure in bulk bins and was measured with the Schleuniger test apparatus (Beumer and Vooijs, 1993). Individual pellets are placed between a moving ram and a flat anvil. The moving ram is pushed against the pellet by an electrical driven spindle with increasing force. The force needed to fracture the pellet is recorded by a force transducer and registered.

Table 1. Composition of experimental diets.

Ingredients (g/kg)	PM-1[1]	PM-5	TM-1	TM-5
Peas	330.4	330.9	0	0
Tapioca	0	0	415.0	415.0
Maize	335.0	330.0	145.0	145.0
Sorghum	0	0	0	0
Wheat	0	0	0	0
Soybeans, extracted	111.4	166.5	274.6	255.7
Rape seed, extracted	44.0	0	0	0
Sunflower seed, extracted	38.5	17.1	21.8	0
Potato protein	0	0	2.4	30.0
Feathermeal, hydrolyzed	20.0	16.8	20.0	20.0
Fish meal	0	31.1	0	34.5
Soybean oil	23.7	37.2	30.5	46.6
Animal fat	59.7	31.8	54.6	20.9
Formic acid	5.0	5.0	5.0	5.0
L-Lysine (25%)	0	0	2.1	0
L-Lysine + DL-methionine (20+10%)	0	0	0	2.7
DL-Methionine (10%)	1.0	3.0	0	0
L-Lysine + L-tryptophane (18+5%)	0	2.6	0	0
Ground limestone	6.2	5.6	2.7	1.7
Monocalcium phosphate	4.2	2.0	5.2	3.0
Sodium chloride	1.0	0.3	1.1	0.2
Vitamin-mineral premix[2]	10.0	10.0	10.0	10.0
Anticoccidial premix[3]	5.0	5.0	5.0	5.0
Phytase premix[4]	5.0	5.0	5.0	5.0
Calculated nutrient composition (g/kg)				
$AME_{broilers}$ (MJ/kg)	12.45	12.44	12.45	12.45
Dry matter[5]	865	864	876	875
Ash	46	46	61	60
Crude Protein[5]	188	213	184	209
Crude Fat	105	94	101	87
Crude Fiber	42	34	41	36
Starch[5]	342	349	342	336
Digestible lysine	8.50	11.01	8.49	10.98
Digestible methionine and cysteine	7.30	8.02	6.69	8.02

[1] PM1 = pea/maize diet with 8.5 g digestible lysine / kg; PM5 = pea/maize diet with 11.0 g digestible lysine / kg; TM1 = tapioca/maize diet with 8.5 g digestible lysine / kg; TM5 = tapioca/maize diet with 11.0 g digestible lysine / kg.

[2] This premix contained the following micro-elements (mg/kg): Mn, 7000; Zn, 3700; Fe, 4500; Cu, 1200; I, 100; Se, 15; vitamin A, 1000000 IU; vitamin D_3, 200000 IU; vitamin E, 2500 IU; menadione, 125; thiamin, 50; riboflavin, 500; pyridoxine, 300; cyanocobalamin, 1.5; nicotinic acid, 4000; folic acid, 100; d-pantothenic acid, 800; choline, 20000; biotin, 10.

[3] This premix contained (mg/kg): Salinomycin-Na, 12000.

[4] This premix contained 100000 FTU per kg.

[5] Analysed values.

Particle Size Distribution. Particle size distribution (Table 2) was determined by wet sieve analysis in which 50 g material was put in a beaker and 250 ml demineralised water was added. After 60 min soaking, the mix of feed and water was stirred for another 60 min at 500 rpm and subsequently put on top of a set of 6 sieves: 2.5, 2.0, 1.4, 1.0, 0.6 and 0.1 mm. Thirty liter of tap water was sprayed through the system, during which the sieves were vibrated with an amplitude of 1 mm (without interruptions). Vibration was stopped 1 min after the tap was closed. Residues on top of each sieve were determined.

Table 2. Particle size distribution and pellet quality of experimental diets, differing in starch sources.

	MPS[1] (mm)	Particle size distribution (%)			Pellet quality		
		>2.0 mm	>1.4 mm	>0.6 mm	Hardness (N)	% Fines	Durability (%)
PM3	0.48	1.6	8.2	32.0	7	5	91
TM3	0.34	0.7	4.5	20.8	7	3	88

[1] MPS = mean particle size; PM3 = pea/maize diet with 9.75 g digestible lysine / kg; TM3 = tapioca/maize diet with 9.75 g digestible lysine / kg.

***In vitro* Starch Digestion.** *In vitro* starch digestion was determined as described by Weurding et al. (2001). In this procedure, which simulates the consecutive digestive processes in the various parts of the broiler alimentary tract, test tubes containing the feed sample, glass balls, a mixture of digestive enzymes and a buffer solution were incubated in a shaking water bath (37°C). After each of nine incubation times (0.25, 0.50, 0.75, 1, 2, 3, 4, 5 and 6 hours), aliquots were taken from the tubes and the amount of released glucose was measured colorimetrically. Starch digestion coefficients (*DC*) were calculated for each incubation time. Rapidly digestible starch (RDS_p), slowly digestible starch (SDS_p) and resistant starch (RS_p) fractions for poultry were calculated from the *DC* as measured after 2 and 4 hours (DC_2 and DC_4). *In vitro* starch digestion after 2 and 4 h correlated best with *in vivo* starch digestion until the posterior jejunum and the posterior ileum respectively (Weurding et al., 2001*b*). RDS_p (%) was defined as $1.16 \cdot DC_2 - 21.5$, SDS_p as $1.29 \cdot DC_4 - 30.9 - RDS$ and RS_p as $100 - RDS_p - SDS_p$. The starch digestion coefficient (*DC*) was plotted against time (*t*) to give an exponential rate curve ($DC_t = D \cdot (1 - e^{-k(d) \cdot t})$ from which the potentially digestible starch fraction (*D*, %) and the starch digestion rate (k_d, /h) were estimated. The *D* fraction represents the asymptote of the digestion curve and the k_d determines the steepness of the curve (a higher k_d means that the curve is steeper).

Statistical Analysis

Data for feed intake, weight gain and feed conversion ratio were analyzed with analysis of variance. The effect of starch source (SS), digestible lysine content (LYS, LYS^2, LYS^3 and LYS^4) and the interaction between these factors on performance were tested according to the following model:

$$Y_{ijkl} = \mu + W9 + BLOCK_i + SS_j + LYS_k + LYS^2_k + LYS^3_k + LYS^4_k + (SS * LYS^*)_{jk} + e_{ijkl}$$

where Y is the weight gain, feed intake or feed conversion ratio; μ is the overall mean; W9 is the covariate, bodyweight at d 9 (start of experiment); BLOCK is block (i = 1, ..., 4); SS is the starch source (j = 1, 2); LYS is the digestible lysine content (k = 1, ..., 5); SS * LYS^* is the interaction between SS and LYS, LYS^2, LYS^3 and LYS^4; and e is the residual error term.

Results

In vitro starch digestion

Diets PM3 and TM3 had an estimated potentially digestible starch fraction of 100% (Table 3). The contrast in starch digestion rate (k_d) between PM3 (1.05 /h) and TM3 (1.99 /h) was as planned. Predicted total starch digestion was high for both diets (98% for PM3 and 104% for TM3). The predicted amount of slowly digestible starch was 183 g/kg starch (61 g/kg feed) for PM3 and 132 g/kg starch (44 g/kg feed) for TM3.

Table 3. Starch characteristics of pea/maize diets and tapioca/maize diets with 9.75 g digestible lysine /kg (PM3 and TM3 respectively)[1].

Diet	Starch (g/kg)	RDS_p (g/kg starch)	SDS_p (g/kg starch)	DS_p (g/kg starch)	D (%)	k_d (/h)
PM3	334	798	183	981	100	1.05
TM3	332	910	132	1042	100	1.99

[1] Potential starch digestibility (D) and fractional starch digestion rate (k_d) were estimated from *in vitro* starch digestion coefficients at different incubation times (DC_t) according to $DC_t = D * (1 - e^{-k(d) \cdot t})$. Rapidly digestible starch (RDS_p), slowly digestible starch (SDS_p) and digestible starch (DS_p) were calculated as explained in the text.

Starch digestion rate, amino acid content and performance

d 9-30. Feed intake was not affected by starch source ($P > 0.05$). For each lysine level, weight gain and FCR were consequently better for birds on pea/maize (PM) diets than for birds on tapioca/maize (TM) diets ($P < 0.01$). Average weight gain (across lysine treatments) of birds on PM and TM diets was 1426 and 1400 g respectively. FCR was on average 1.55 and 1.58 for birds on PM and TM diets respectively. Adding digestible lysine to the diet reduced feed intake both linearly ($P < 0.01$) and quadratically ($P < 0.10$) from 2245 to 2193 g. Weight gain increased linearly from 1397 to 1431 g in diets with higher digestible lysine contents ($P < 0.01$) and FCR decreased both linearly ($P < 0.01$) and quadratically ($P < 0.10$) from 1.608 to 1.533 in diets with increasing digestible lysine contents. No significant interaction between starch source and digestible lysine content was observed for weight gain. However, FCR data pointed to an interaction between starch source and digestible lysine content ($P = 0.11$).

Table 4. Effect of starch source (SS) and digestible lysine content (LYS) on performance of broiler chickens.

		SS	Digestible lysine content (g/kg)					SEM	Effect			
			8.50	9.13	9.75	10.38	11.00		SS	LYS	LYS^2	SS x LYS
d 9-30	Weight gain, g	PM[1]	1417	1406	1425	1437	1444	9	**	**	NS	NS
		TM	1376	1395	1400	1413	1418					
	Feed intake, g	PM	2248	2206	2198	2204	2200	10	NS	**	†	NS
		TM	2241	2231	2201	2212	2186					
	FCR	PM	1.586	1.569	1.542	1.534	1.524	0.006	**	**	†	0.11
		TM	1.629	1.599	1.573	1.565	1.543					
d 9-18	Weight gain, g	PM	463	468	476	477	483	3	**	**	0.12	NS
		TM	452	467	469	472	477					
	Feed intake, g	PM	655	646	646	639	644	4	**	**	†	NS
		TM	668	669	655	648	655					
	FCR	PM	1.414	1.379	1.356	1.339	1.334	0.007	**	**	**	*
		TM	1.478	1.432	1.398	1.375	1.374					
d 18-30	Weight gain, g	PM	954	938	948	959	961	8	**	*	NS	NS
		TM	924	928	931	942	941					
	Feed intake, g	PM	1595	1562	1554	1569	1558	8	†	**	NS	NS
		TM	1574	1564	1548	1567	1533					
	FCR	PM	1.672	1.666	1.638	1.635	1.621	0.010	**	**	NS	NS
		TM	1.704	1.686	1.663	1.664	1.630					

[1] PM = pea/maize diet; TM = tapioca/maize diet.
** = $P < 0.01$; * = $P < 0.05$; † = $P < 0.10$; NS = not significant ($P > 0.10$); $n = 4$.

d 9-18. Feed intake was lower (646 vs 659 g) and weight gain higher (474 vs. 467 g) for birds on PM diets than those on TM diets ($P < 0.01$). Adding digestible lysine to the diet reduced feed intake linearly ($P < 0.01$) and quadratically ($P < 0.10$) from 662 to 649 g and increased weight gain linearly ($P < 0.01$) from 458 to 480 g. An interaction between starch source and digestible lysine content on FCR was observed ($P < 0.05$). FCR was lower for birds on PM diets than those on TM diets and the difference was most pronounced with lower digestible lysine contents in the diets.

d 18-30. Feed intake ($P < 0.10$) and weight gain ($P < 0.01$) were higher and FCR was lower ($P < 0.01$) for birds on PM diets than those on TM diets. Adding digestible lysine to the diet reduced feed intake ($P < 0.01$), increased weight gain ($P < 0.05$) and decreased FCR ($P < 0.01$) linearly.

Discussion

The positive effect of slowly digestible starch on performance was clearly confirmed in this experiment. From Table 4 it is clear that birds on pea/maize diets grew faster and more efficient than those on tapioca/maize diets. Within starch treatments, feed intake was higher for birds on the diets with 8.5 g/kg digestible lysine compared to diets with higher levels of digestible lysine. This effect appeared in the second period (d 18-30) and was also observed in the whole experimental period. A curvilinear relation between digestible lysine content and feed intake was observed from d 9-30. Feed intake of birds on 10.38 g/kg digestible lysine from d 18-30 was higher than expected and therefore no quadratic effect was observed. From d 9-18, feed intake of birds on 10.38 g/kg digestible lysine was slightly less than expected. A tendency to an interaction between starch source (starch digestion rate) and digestible lysine content was observed for FCR over the whole experimental period ($P = 0.11$). This interaction was significant when measured from d 9-18. No interaction between these effects was observed for weight gain. Weurding et al. (Chapter 6) observed a similar interaction between starch digestion rate and digestible amino acid content on FCR of broiler chickens for untreated and expander treated peas and maize during the grower phase (d 14-30; $P < 0.05$). However, during the starter phase (d-14), an inverse interaction was observed in that experiment ($P = 0.07$) and therefore, the interaction did not reach significance ($P = 0.11$) when expressed over the whole experimental period (d 0-39). Birds on diets with slowly digestible starch had a lower FCR than birds on diets with rapidly digestible starch. Because the difference is more pronounced for birds on diets with low amino acid levels, this indicates that amino acid supply and glucose supply are unbalanced in diets with rapidly digestible starch.

This may mean that a diet with synchronized starch and protein digestion (e.g. a more gradual starch digestion) results in better performance. The explanation may be found in hormonal responses to glucose absorption which affect protein deposition. A gradual starch digestion results in a lower, but longer lasting insulin peak than a rapid starch digestion. Elevated insulin levels are required for amino acid transport and uptake by body cells (Fox, 1996). On the other hand, asynchrony of starch and protein digestion may increase the oxidation of amino acids to meet the energy demand of gut tissues. When glucose is metabolized in the gut tissues of the posterior part of the small intestine, as may be the case when diets with slowly digestible starch are fed, amino acids may be spared and can thus be used for muscle growth.

Both weight gain and FCR improved with higher digestible lysine contents in the diet. Although 11.0 g/kg digestible lysine is well above the normal requirement for growing broiler chickens, the lysine requirement may have been higher for the birds in this experiment. Some leg problems occurred in the preliminary period. These were associated with a growth depression of the birds. After a quick recovery following the supply of an additional vitamin-mineral mix via water bowls, the birds compensated for the growth depression and therefore required more lysine than in a normal situation. This can explain that weight gain and FCR were still improving with the last lysine step. As a result it is not possible to quantify the amino acid sparing effect of slowly digestible starch accurately. On the other hand, due to the higher lysine deficiency in this situation, interaction between starch digestion rate and amino acid requirement is more likely to be demonstrated. Our data suggest that the plateau value for FCR is not the same for birds on pea/maize and tapioca/maize diets. This implies that the improved protein efficiency due to slowly digestible starch is not the only factor explaining the improved feed efficiency. Energy efficiency may also be improved by feeding slowly digestible starch. A more or less continuous glucose supply enables more direct utilization of glucose for processes in the body. Less energy consuming conversions of glucose to glycogen or fat and back to glucose are needed in that situation. Based on the results of this experiment we conclude that feeding slowly digestible starch improves feed efficiency compared to feeding rapidly digestible starch. The major part of this improvement can be attributed to an improved protein utilization. The protein sparing effect can not explain the total improvement. It is likely that energy utilization is also improved because of the prolonged elevated plasma glucose levels.

Chapter 8

Broiler Performance as Affected by Varying Levels of Starch Digestion Rate

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Abstract

Two experiments were conducted to study the effect of varying levels of starch digestion rate (k_d) on broiler performance. In both experiments, two diets were formulated with feedstuffs containing either slowly digestible (SDS) or rapidly digestible starch (RDS). These diets were mixed in various proportions in order to obtain diets with increasing levels of k_d. In experiment 1, an additional diet was formulated which contained SDS from other feedstuffs than the other SDS diet. In experiment 2, an additional diet was produced which contained RDS from processed feedstuffs. These additional diets were included to test whether effects of k_d on performance were independent of the specific starch source. In experiment 1, in vitro starch digestion rate varied from 0.9 to 2.0 / h. FCR (d 9-30) of the birds on the RDS diet was 1.57 and FCR of both SDS diets was 1.54 ($P < 0.05$). In experiment 2, in vitro starch digestion rate varied from 0.7 to 2.1 / h. FCR (d 7-30) of the birds on the two RDS diets (1.8 and 2.1 /h) was 1.57 and FCR of the birds on the SDS diet (0.7 / h) was 1.51 ($P < 0.05$). FCR of birds on diets with intermediate k_d levels was in between these extremes. Starch digestion rate did not affect levels of Clostridium perfringens or Lactobacilli in caecal contents ($P > 0.05$). From these experiments it was concluded that within the range of 0.8 to 1.8 / h, a slow starch digestion improves feed efficiency of broiler chickens.

Keywords: starch digestion rate, performance, broiler chickens, *Clostridium perfringens, Lactobacilli*

Introduction

Feed evaluation systems for broiler chickens are based on digested nutrients. Sofar, the kinetics of digestion are not incorporated in feed evaluation systems for broiler chickens. The rate at which polymers (carbohydrates, proteins) are broken down to absorbable monomers (glucose and amino acids) and dimers (peptides) determine both the time and site of absorption. Time of absorption, relative to time of feed intake, is important for a simultaneous availability of energy yielding nutrients and amino acids. Amino acid uptake by body cells requires insulin (Fox, 1996). Insulin is secreted as a response to elevated plasma glucose levels after feed intake. A more or less continuous glucose release from the small intestine prolongs the time of elevated insulin levels and favors amino acid uptake by body cells. Furthermore, a continuous glucose release may also result in improved energy efficiency. In addition, site of absorption may have consequences for the fate of the absorbed nutrients. Absorption of nutrients coincides with their partial catabolism in the gut wall (Vaugelade et al., 1993). Amino acids and glucose may be oxidized in the gut wall in order to meet the energy demand of this active organ. A shortage of the preferred nutrient may result in oxidation of another nutrient. A diet with rapidly digestible starch only provides the upper small intestine with dietary glucose, whereas a diet with slowly digestible starch supplies the lower small intestine with dietary glucose as well. When no dietary glucose is available for the energy supply of the lower small intestine, more amino acids may be oxidized for this purpose. This may impair the protein efficiency of the birds in situations when the supply of limiting amino acids is not excessive.

There is evidence now that site or rate of starch digestion can affect performance of broiler chickens (Weurding et al., Chapters 5 and 6). Experiments pointed out that broiler diets containing starch with a low digestion rate improved weight gain and feed efficiency compared to diets containing starch with a high digestion rate. Feeding slowly digestible starch appears to increase both protein and energy efficiency of broiler chickens. The relationship between the level of starch digestion rate and performance of broiler chickens is not known. Therefore, the primary objective of the experiments described in this paper was to relate the level of starch digestion rate, that can be found in practical broiler diets, to broiler performance. Two dose-response experiments have been carried out in which different levels of starch digestion rate were incorporated in the experimental diets.

In our previous experiments, slowly digestible starch (SDS) was always obtained by high dietary pea levels and in most cases, rapidly digestible starch (RDS) was obtained by high dietary tapioca levels. Therefore, an SDS diet without peas (experiment 1) and an RDS diet without tapioca (experiment 2) were included in the current experiments to test whether effects of starch digestion rate on performance were independent of the specific starch source.

Feeding diets with slowly digestible starch may increase the amounts of resistant starch (RS) which enters the hind gut (Björck et al., 2000; Weurding et al., 2001a). The caeca of broiler chickens serve as the principal site for microbial fermentation of complex carbohydrates that resist digestion in the small intestine (Klasing, 1998). Higher amounts of resistant starch in the material entering the caeca may shift microbial populations in favor of species that thrive well on starch. Weurding et al. (Chapter 6) observed that diets with more resistant starch led to lower amounts of *Clostridium perfringens* bacteria without changes in *Lactobacilli* bacteria. This is regarded as a positive effect because high numbers of *Clostridium perfringens*, which thrive well on protein (Van der Steen et al., 1997), are associated with a high risk of necrotic enteritis (Kaldhusdal et al., 1999). It is important to find effective means to stimulate a healthy microbial population in the broiler alimentary tract. One way is to manipulate the type of substrate entering the hind gut, which is referred to as the prebiotic concept. In experiment 1, the effect of different levels of starch digestion rate on bacterial counts of *Clostridium perfringens* and *Lactobacilli* in the caeca was investigated.

Materials and methods

Animals and Housing

Experiment 1. The experiment was performed with 2720 one-day old male and female broiler chickens of the Cobb 500 strain. Chicks were obtained from Cobroed, Lievelde, The Netherlands, and were housed in two compartments. For this experiment, eight pens per compartment were used and in each pen 85 male and 85 female chickens were housed. Both compartments were divided in two blocks of four pens each. This experiment was jointly performed with another experiment in which the response of birds to varying amino acid levels was investigated at two levels of starch digestion rate (Weurding et al., Chapter 7). From d 0-9, all chicks were fed the same starter diet containing peas, tapioca and maize. From d 9-30, the birds were fed one of four experimental diets, which were randomly assigned to a pen in each block. Average bodyweight at the start of the experimental period (d 9) was 229 g.

Experiment 2. This experiment was performed with 840 one-day-old female broiler chickens of the Cobb 500 strain (Cobroed, Lievelde). Chicks were housed in a building with 24 floor pens. The building was divided in four blocks, each with six floor pens. An experimental unit was formed by one floor pen, containing 35 birds. From d 0-7, all chicks were fed the same starter diet containing sorghum, peas, tapioca and maize as starch sources. From d 7-30, the birds were fed one of six experimental diets, which were randomly assigned to a pen in each block. Average bodyweight at the start of the experimental period (d 7) was 145 g.

In both experiments, a 23 h light and 1 h dark interval was used and chicks had unrestricted access to feed and water. The experimental protocols were in agreement with the standards for animal experiments and were approved by the Ethical Committee of 'De Schothorst'.

Diets

Experiment 1. Based on previous analyses on different starch sources, peas and sorghum (slowly digestible starch), maize and wheat (gradually digestible starch) and tapioca (rapidly digestible starch) were used in this experiment. Three grower diets were formulated. One diet contained 50% sorghum and 6% wheat (SW), one contained 35% peas and 35% maize (PM) and one contained 42% tapioca and 15% maize (TM). The fourth diet was a 50/50 mix of the PM and TM diet (PTM). Dietary composition is given in Table 1. Diets were conditioned with steam at 55-58°C for ± 10 s and subsequently pelleted through a 3 x 80 mm die (exit temperature of pellets varied from 58-62°C).

Table 1. Composition of experimental diets in experiment 1.

Ingredients (g/kg)	SW[1]	PM	PTM	TM
Peas	0.0	330.5	165.2	0.0
Tapioca	0.0	0.0	207.5	415.0
Maize	0.0	332.5	238.7	145.0
Sorghum	500.0	0.0	0.0	0.0
Wheat	64.4	0.0	0.0	0.0
Soybeans, extracted	190.7	139.0	202.0	265.1
Rape seed, extracted	44.0	22.0	11.0	0.0
Sunflower seed, extracted	76.1	27.8	19.3	10.9
Potato protein	3.0	0.0	8.1	16.2
Feathermeal, hydrolyzed	0.0	18.4	19.2	20.0
Fish meal	3.5	15.5	16.3	17.2
Soybean oil	26.5	30.5	34.5	38.5
Animal fat	44.0	45.7	41.7	37.7
Formic acid	5.0	5.0	5.0	5.0
L-Lysine (25%)	8.0	0.0	0.5	1.0
Lysine + methionine (20+10%)	0.0	0.0	0.6	1.3
DL-Methionine (10%)	2.6	2.0	1.0	0.0
Lysine + tryptophane (18+5%)	0.0	1.3	0.6	0.0
Ground limestone	7.4	5.9	4.0	2.2
Monocalcium phosphate	3.7	3.1	3.6	4.1
Sodium chloride	1.1	0.6	0.6	0.6
Vitamin-mineral premix[2]	10.0	10.0	10.0	10.0
Anticoccidial premix[3]	5.0	5.0	5.0	5.0
Phytase premix[4]	5.0	5.0	4.9	4.8
Calculated nutrient composition (g/kg)				
$AME_{broilers}$ (MJ/kg)	12.45	12.45	12.44	12.44
Dry matter[5]	881	863	870	872
Ash	50	46	53	60
Crude Protein[5]	212	199	199	194
Crude Fat	94	100	97	94
Crude Fiber	43	38	38	38
Starch[5]	344	344	347	340
Digestible lysine	9.7	9.7	9.7	9.7
Digestible methionine and cysteine	7.9	7.7	7.5	7.4

[1] SW = sorghum/wheat diet; PM = pea/maize diet; PTM = pea/tapioca/maize diet and TM = tapioca/maize diet.
[2] This premix contained the following micro-elements (mg/kg): Mn, 7000; Zn, 3700; Fe, 4500; Cu, 1200; I, 100; Se, 15; vitamin A, 1000000 IU; vitamin D_3, 200000 IU; vitamin E, 2500 IU; menadione, 125; thiamin, 50; riboflavin, 500; pyridoxine, 300; cyanocobalamin, 1.5; nicotinic acid, 4000; folic acid, 100; d-pantothenic acid, 800; choline, 20000; biotin, 10.
[3] This premix contained (mg/kg): Salinomycin, 12000.
[4] This premix contained 100000 FTU phytase per kg.
[5] Analysed values.

Experiment 2. Two grower diets were formulated: one diet contained tapioca and maize (TM) with a high starch digestion rate and one diet contained sorghum and peas (SP) with a low starch digestion rate. These two diets were mixed in varying proportions (100/0, 75/25, 50/50, 25/75 and 0/100) to obtain five diets with increasing starch digestion rate (SP-100 to SP-0). Sub-batches of the sorghum and peas were puffed according to the Presco® expansion procedure of Meneba Feed Ingredients (Weert, The Netherlands) in order to increase starch digestion rate. These feedstuffs were incorporated in the sixth experimental diet (SP-P). Dietary composition is given in

Table 2. Diets were steam-conditioned at 38-43 °C for ± 10 s and subsequently pelleted through a 3 x 80 mm die (exit temperature of pellets varied from 59-62 °C).

Analysis

Diets were analysed for dry matter, nitrogen (Dumas), starch (Brunt et al., 1998) and *in vitro* starch digestion (Weurding et al., 2001*b*). In addition pellet quality was measured by means of durability, hardness and percentage of fines as described by Weurding et al. (Chapter 7). Pellet quality may affect feed intake. Particle size distribution (PSD) was determined for each diet in experiment 1 and for diets SP-0, SP-100 and SP-P in experiment 2. This was done because the size of feed particles may affect digestibility. PSD was determined by wet sieve analysis as described by Weurding et al. (Chapters 6 and 7). Data on PSD and pellet quality are presented in Table 3.

At d 29 of experiment 1, three hens were removed from each pen of treatment PM and TM. These 24 birds were euthanised by an intravenous injection of T61, this is an aqueous solution containing 200 g embutramide, 50 g mebezoniumiodide and 5 g tetracainehydrochloride per liter (Hoechst Veterinär GmbH, München, Germany). Immediately after injection, caeca were removed from the dead bird and caeca contents were gently squeezed out and prepared for bacterial counts of *Clostridium perfringens* (NCFA, 1997) and *Lactobacilli* (Smits et al., 1998).

Statistical Analysis

Data for feed intake, weight gain and feed conversion ratio (FCR) were analysed with analysis of variance. Treatment effects were tested according to the following model:

$$Y_{ijk} = \mu + Ws + BLOCK_i + TREAT_j + e_{ijk}$$

where Y is the weight gain, feed intake or feed conversion ratio, μ is the overall mean, Ws is the bodyweight at the start of experiment (d 9 or d 7), BLOCK is block (i = 1, ..., 4), TREAT is the treatment (j = 1, ..., 4 for exp. 1 and j = 1, ..., 6 for exp. 2), and *e* is the residual error term.

Bodyweight at d 9 (experiment 1) or 7 (experiment 2) was used as a covariate (Ws) and block effects were incorporated in the statistical model. Differences in bacterial counts in experiment 1 were tested with block and treatment (PM or TM) as main effects in the model: $Y_{ijk} = \mu + BLOCK_j + TREAT_j + e_{ijk}$. Data were analysed with the general linear models procedure of SAS (SAS Institute, 1989).

Table 2. Composition of experimental diets in experiment 2.

Ingredients (g/kg)	SP-100	SP-75	SP-50	SP-25	SP-0	SP-P
Sorghum	394.5	295.8	197.2	98.6	0.0	394.5
Peas	271.9	203.9	135.9	67.9	0.0	271.9
Tapioca	0.0	105.8	211.7	317.6	423.5	0.0
Maize	0.0	46.2	92.5	138.7	185.0	0.0
Soybeans, extracted	174.0	180.8	187.7	194.6	201.5	174.0
Rape seed, extracted	40.0	30.0	20.0	10.0	0.0	40.0
Potato protein	0.0	7.5	15.0	22.5	30.0	0.0
Feathermeal, hydrolyzed	0.0	5.0	10.0	15.0	20.0	0.0
Fish meal	0.0	3.8	7.7	11.6	15.5	0.0
Blood meal	0.0	7.0	14.0	21.0	28.0	0.0
Soybean oil	39.4	37.8	36.2	34.6	33.0	39.4
Animal fat	30.0	30.2	30.5	30.7	31.0	30.0
Formic acid	5.0	5.0	5.0	5.0	5.0	5.0
L-Lysine (25%)	2.2	1.6	1.1	0.5	0.0	2.2
Lysine + methionine (20+10%)	1.1	0.8	0.5	0.2	0.0	1.1
DL-Methionine (10%)	1.0	0.7	0.5	0.2	0.0	1.0
Threonine (10%)	9.6	7.2	4.8	2.4	0.0	9.6
Ground limestone	6.3	5.3	4.4	3.4	2.5	6.3
Monocalcium phosphate	4.2	4.3	4.5	4.7	4.9	4.2
Sodium chloride	0.8	0.6	0.4	0.2	0.1	0.8
Vitamin-mineral premix[1]	10.0	10.0	10.0	10.0	10.0	10.0
Anticoccidial premix[2]	5.0	5.0	5.0	5.0	5.0	5.0
Phytase premix[3]	5.0	5.0	5.0	5.0	5.0	5.0
Calculated nutrient composition (g/kg)						
$AME_{broilers}$ (MJ/kg)	12.46	12.45	12.46	12.45	12.46	12.46
Dry matter[4]	894	889	886	888	886	882
Ash	47	49	51	54	56	47
Crude Protein[4]	211	210	206	201	200	203
Crude Fat	89	87	84	82	80	89
Crude Fiber	37	37	37	36	36	37
Starch[4]	366	359	366	371	372	354
Digestible lysine	10.0	10.0	10.0	10.0	10.0	10.0
Digestible methionine and cysteine	7.3	7.3	7.3	7.3	7.4	7.3

[1] This premix contained the following micro-elements (mg/kg): Mn, 7000; Zn, 3700; Fe, 4500; Cu, 1200; I, 100; Se, 15; vitamin A, 1000000 IU; vitamin D_3, 200000 IU; vitamin E, 2500 IU; menadione, 125; thiamin, 50; riboflavin, 500; pyridoxine, 300; cyanocobalamin, 1.5; nicotinic acid, 4000; folic acid, 100; d-pantothenic acid, 800; choline, 20000; biotin, 10.
[2] This premix contained (mg/kg): Lasalocid, 18000.
[3] This premix contained 100000 FTU phytase per kg.
[4] Analysed values.

Results

In vitro starch digestion

Experiment 1. The estimated potentially digestible starch fraction was 100% for all diets (Table 4). *In vitro* starch digestion rate (k_d, fraction /h) varied from 0.86 to 1.99 /h between diets. Predicted total starch digestion varied from 981 for PM to 1042 g/kg starch for TM. The predicted amount of slowly digestible starch varied from 132 to 266 g/kg starch (corresponding to 44 to 91 g/kg feed).

Table 3. Particle size distribution and pellet quality of experimental diets.

	MPS (mm)	Particle size distribution (%)			Pellet quality		
		>2.0 mm	>1.4 mm	>0.6 mm	Hardness (N)	% Fines	Durability (%)
Experiment 1							
SW[1]	0.39	0.8	3.2	25.2	8	2	89
PM	0.48	1.6	8.2	32.0	7	5	91
PTM	0.42	1.5	7.1	27.2	7	1	89
TM	0.34	0.7	4.5	20.8	7	3	88
Experiment 2							
SP-100	0.37	0.7	3.2	25.0	6	9	83
SP-75	-[2]	-	-	-	8	4	88
SP-50	-	-	-	-	8	2	89
SP-25	-	-	-	-	9	2	90
SP-0	0.29	0.3	2.4	17.0	9	1	91
SP-P	0.27	0.1	1.7	13.8	11	1	96

[1] SW = sorghum/wheat diet; PM = pea/maize diet; PTM = pea/tapioca/maize diet; TM = tapioca/maize diet and SP = sorghum/pea diet.
[2] = not determined.

Experiment 2. The estimated potentially digestible starch fraction was 100% for all diets. *In vitro* starch digestion rate (k_d) of diet SP-100 (0.73 /h) was substantially lower than that of diet SP-0 (1.82 /h). The diet with puffed sorghum and peas (SP-P) had a k_d of 2.06 /h. The mixed diets had k_d values that were in between that of diets SP-100 and SP-0. Total starch digestion was predicted to vary from 913 (SP-100) to more than 1000 g/kg starch for diets SP-50, SP-25 and SP-0. The predicted amount of slowly digestible starch varied from 86 to 242 g/kg starch (corresponding to 31 to 84 g/kg feed).

Table 4. Starch characteristics of experimental diets[1].

Diet	Starch (g/kg)	RDS_p (g/kg starch)	SDS_p (g/kg starch)	DS_p (g/kg starch)	*D* (%)	k_d (/h)
Experiment 1						
SW[2]	344	725	266	991	100	0.86
PM	334	798	183	981	100	1.05
PTM	333	880	150	1029	100	1.43
TM	332	910	132	1042	100	1.99
Experiment 2						
SP-100	348	671	242	913	100	0.73
SP-75	346	739	220	959	100	0.95
SP-50	350	815	186	1000	100	1.28
SP-25	356	855	156	1011	100	1.50
SP-0	360	911	108	1019	100	1.82
SP-P	360	888	86	974	99	2.06

[1] Potential starch digestibility (*D*) and fractional starch digestion rate (k_d) were estimated from *in vitro* starch digestion coefficients at different incubation times (DC_t) according to $DC_t = D * (1 - e^{-k(d) \cdot t})$. Rapidly digestible starch (RDS_p), slowly digestible starch (SDS_p) and digestible starch (DS_p) were calculated as explained by Weurding et al. (Chapter 7).
[2] SW = sorghum/wheat diet; PM = pea/maize diet; PTM = pea/tapioca/maize diet; TM = tapioca/maize diet and SP = sorghum/pea diet.

Starch digestion rate and performance

Experiment 1. Weight gain was not significantly ($P > 0.05$) affected by the dietary k_d level when measured over the experimental period (Table 5). Birds on the SW diet, which had the lowest k_d, had a lower feed intake than those on the other diets ($P < 0.05$). FCR was better for birds on diets with a slow starch digestion than for birds on diets with a rapid starch digestion ($P < 0.05$). FCR of birds on SW and PM diets was better than FCR of birds on the TM diet. Weight gain and FCR were best for birds on the PM diet and worst for birds on the TM diet.

Table 5. Bird performance as effected by diets with different starch sources (experiment 1).

Diet		SW[1]	PM	PTM	TM	SEM	LSD
k_d (/h)		0.86	1.05	1.43	1.99		
d 9-30	weight gain, g	1404	1425	1415	1400	8	25
	feed intake, g	2157^b	2197^a	2198^a	2201^a	11	36
	FCR	1.537^c	1.542^{bc}	1.553^b	1.572^a	0.005	0.015
d 9-18	weight gain, g	463^c	476^a	475^{ab}	468^{bc}	2	7
	feed intake, g	634^b	645^{ab}	647^{ab}	654^a	4	12
	FCR	1.369^b	1.356^b	1.361^b	1.398^a	0.005	0.018
d 18-30	weight gain, g	941	949	940	932	7	22
	feed intake, g	1527^b	1554^a	1554^a	1549^{ab}	8	26
	FCR	1.623^c	1.637^{bc}	1.654^{ab}	1.662^a	0.007	0.023

[1] SW = sorghum/wheat diet; PM = pea/maize diet; PTM = pea/tapioca/maize diet; TM = tapioca/maize diet; LSD = least significant difference; FCR = feed conversion ratio (feed intake / weight gain).
[a,b,c] Means within a row without a common superscript differ ($P < 0.05$). $n = 4$.

During the first 9 days of the experimental period, weight gain was best for the birds on diets with intermediate k_d-values and feed intake was higher for birds on diets with a high k_d than for birds on diets with low k_d. FCR was lower for birds on PTM, PM and SW diets than for those on the TM diet. No differences in weight gain were observed during the last 12 days of the experimental period. Feed intake of birds on the SW diet was lower than for the birds on the other diets. FCR was best for birds on diets with low k_d-values ($P < 0.05$).

Experiment 2. Feed intake was low for birds on diets with a slow starch digestion compared to birds on diets with a rapid starch digestion ($P < 0.05$). Weight gain was less affected than feed intake by starch digestion rate. Therefore, feed conversion ratio of the birds was lower for birds on diets with a slow starch digestion ($P < 0.05$). These effects were seen throughout the whole experimental period. FCR of birds on diets SP-P and SP-0 was the same. FCR of birds on diet SP-100 (slow starch digestion) was lower than FCR of birds on diets SP-0 and SP-P (rapid starch digestion).

Table 6. Bird performance as affected by diets with different starch sources (experiment 2).

Diet		SP-100[1]	SP-75	SP-50	SP-25	SP-0	SP-P	SEM	LSD
k_d (/h)		0.73	0.95	1.28	1.50	1.82	2.06		
d 7 – 30	weight gain, g	1219^{b}	1231^{ab}	1233^{ab}	1236^{ab}	1258^{a}	1243^{ab}	10	30
	feed intake, g	1833^{e}	1884^{d}	1911^{cd}	1922^{bc}	1971^{a}	1949^{ab}	10	30
	FCR	1.505^{c}	1.531^{bc}	1.550^{ab}	1.555^{ab}	1.567^{a}	1.568^{a}	0.009	0.026
d 7 – 17	weight gain, g	403	412	412	415	415	407	4	12
	feed intake, g	521^{b}	540^{a}	546^{a}	552^{a}	557^{a}	548^{a}	6	17
	FCR	1.294^{c}	1.310^{bc}	1.326^{ab}	1.330^{ab}	1.342^{a}	1.345^{a}	0.008	0.023
d 17 – 30	weight gain, g	816^{b}	818^{b}	821^{ab}	821^{ab}	843^{a}	836^{ab}	7	23
	feed intake, g	1313^{d}	1344^{c}	1366^{bc}	1371^{b}	1414^{a}	1401^{a}	8	25
	FCR	1.611^{b}	1.643^{ab}	1.663^{a}	1.669^{a}	1.678^{a}	1.676^{a}	0.012	0.035

[1] SP = sorghum/pea diet; LSD = least significant difference; FCR = feed conversion ratio (feed intake / weight gain).

^{a,b,c,d,e} Means within a row without a common superscript differ ($P < 0.05$). $n = 6$.

Starch digestion rate and microbial counts

No significant differences in colony-forming units (cfu) were observed for *Clostridium perfringens* or *Lactobacilli* between PM and TM ($P > 0.05$; Figure 1). On average, caecal contents of the birds on the PM and TM diets contained $1.1 \cdot 10^4$ and $1.5 \cdot 10^5$ cfu of *Clostridia perfringens* per gram. Caecal contents of birds on PM and TM diets contained $2.9 \cdot 10^8$ and $2.2 \cdot 10^8$ cfu of *Lactobacilli* per gram.

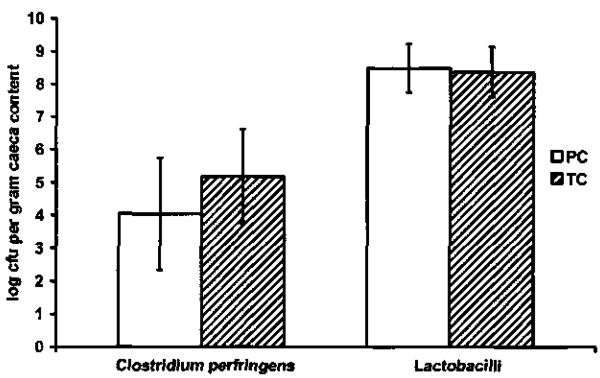

Figure 1. Log cfu *Clostridium perfringens* and *Lactobacilli* found per gram caeca content in broiler chickens fed either a pea/maize (PM) or a tapioca/maize (TM) diet (± SD). $n = 3$ for TM and $n = 4$ for PM.

Discussion

The positive effect of slowly digestible starch on performance as shown in previous studies (Weurding et al., Chapters 5 and 6) was clearly confirmed in these two experiments. In both experiments, diets with a slow *in vitro* starch digestion resulted in a better FCR of the broiler chickens than diets with a rapid *in vitro* starch digestion. In experiment 1, the birds on the pea/maize diet grew faster than the birds on the tapioca/maize diet ($P < 0.10$). No differences in feed intake were observed between these two treatments and therefore FCR was better for birds on the pea/maize diet than for birds on the tapioca/maize diet (1.54 and 1.57 respectively; $P < 0.05$). The birds on the sorghum/wheat diet consumed less feed than the birds on the other three diets ($P < 0.05$), but weight gain was similar to that of the birds on the tapioca/maize diet. Because of this the FCR of birds on the sorghum/wheat diet was better than that of the birds on the tapioca/maize diet (1.54 and 1.57 respectively).

In experiment 2, pellet quality was positively correlated with starch digestion rate. Milling and pelleting conditions were the same for all feeds, therefore the pellet quality was affected by differences in feed composition. The better pellet quality of the feeds with a high starch digestion rate may have caused the higher feed intakes of the birds consuming these diets. This higher feed intake probably led to extra weight gain of these birds. In experiment 1, pellet quality was similar between treatments and differences in feed intake were small. In both experiments, diets with a slower starch digestion resulted in better feed efficiency. These observations, combined with the results of two earlier trials with mash feeds in which birds on slowly digestible starch diets had a better feed efficiency than birds on rapidly digestible starch diets (Weurding et al., Chapters 5 and 6), indicate that the differences in FCR in experiment 2 can mainly be attributed to differences in starch digestion rate.

In our earlier experiments in which the positive effect of slowly digestible starch on performance was demonstrated, slowly digestible starch was always provided by peas. This raised the question whether the positive effect was truly a k_d- or a specific pea effect. In experiment 1, both the pea/maize diet and the sorghum/wheat diet (both contained slowly digestible starch) resulted in a better FCR than the tapioca/maize diet. This is the first time that a slowly digestible starch diet without peas was compared to a rapidly digestible starch diet, and the positive effect of slowly digestible starch was confirmed. In most of our earlier experiments, tapioca was used as a source for rapidly digestible starch. In one experiment a diet containing expander treated peas and maize was used as a rapidly digestible starch diet and feed efficiency of birds on this diet was worse than that of birds on slowly digestible starch diets containing either native or steam pelleted peas and maize (Weurding et al., Chapter 6). In experiment 2, puffed sorghum and maize were used as providers for rapidly digestible starch and birds receiving this diet had a considerably higher FCR than that of birds on the slowly digestible starch diet containing native sorghum and maize. Moreover, FCR of birds on the puffed sorghum/maize diet was similar as FCR of the birds on the tapioca/maize diet. Therefore it can be concluded that the positive effects of slowly digestible starch in our former experiments were not specific pea effects. Neither were the negative effects of rapidly digestible starch specific tapioca effects.

An increase in the level of starch digestion rate from 1.05 (PM) to 1.99 /h (TM) in the diets of experiment 1 resulted in lower weight gain and a higher FCR of birds from d 9-

30. The birds on a diet with a starch digestion rate of 0.86 / h (SW) had a lower weight gain and a similar FCR to that of birds on the PM diet.

During the first part of the experimental period, FCR was slightly higher in the SW group, suggesting that young birds have more problems digesting slowly digestible starch. Yutste et al. (1991) observed that digestion of several starches improved from 2-3 weeks in broiler chickens. This may be explained by the fact that digestive capacity is still increasing in the young bird (Nitsan et al., 1991; Uni et al., 1995). From d 18-30, a positive relationship between k_d level and FCR was observed, indicating that a slower starch digestion improves FCR over the whole experimental range. This effect was also seen from d 9-30.

In experiment 1, the decreasing effect of slowly digestible starch on FCR seems to flatten out at lower digestion rates, suggesting the existence of an optimal k_d level for broiler chickens which is below the investigated range of k_d. In experiment 2, the effect of starch digestion rate was small at high k_d levels, but became bigger at lower levels. This indicates that beyond a certain k_d level, differences in k_d do not affect bird performance anymore because the amount of slowly digestible starch is too low in these circumstances. These observations suggest that the effect of slowly digestible starch on FCR can be described by a sigmoid curve. FCR is not affected by high k_d levels (>1.8 /h), but below this threshold level, a lower k_d results in a better FCR. When starch digestion rate falls below a critical level (<0.8 /h), then FCR will not improve and may even increase because total starch digestion may be impaired.

Consumption of diets with slowly digestible starch results in a different composition of digesta reaching the hind gut than consumption of diets with rapidly digestible starch. This different substrate supply may affect the microbial populations in the hind gut. There may be a shift from protein degrading bacteria to starch degrading bacteria in the hind gut. *Clostridium perfringens* are known to grow on protein rich diets (Van der Steen et al., 1997). No significant differences in colony forming units of *Clostridium perfringens* in caeca of birds fed diets containing slowly digestible or rapidly digestible starch were observed in this experiment. In a former experiment, birds fed a diet containing slowly digestible starch had significantly less colony forming units of *Clostridium perfringens* than birds fed a diet containing rapidly digestible starch (Weurding et al., Chapter 6). The level of *Clostridium perfringens* in the current experiment was higher and the difference between treatments was lower compared to the former experiment. The high level of *Clostridium perfringens* in this experiment (Figure 1) may be caused by the absence of an antibiotic growth promoter in the diets, whereas avilamycin was used in the previous experiment. Elwinger et al. (1998) showed that avilamycin reduced the colony forming units of *Clostridium perfringens* considerably in broiler chickens. The smaller difference in colony forming units of *Clostridium perfringens* between treatments in the current experiment compared to that in Chapter 6 is undoubtedly related to the smaller contrast in starch digestion rate. Starch digestion rate in the pea/maize treatment in the previous experiment was lower (0.84 /h) than in the pea/maize treatment in the current experiment (1.05 /h). In the previous experiment (Chapter 6) the slowly digestible starch diets were not pelleted and pelleting increases starch digestion rate (Weurding et al., Chapter 6). And because starch digestion rate is inversely related to the amount of resistant starch in the diet, this difference will also be reflected in the resistant starch fraction. In fact, *in vitro* predicted resistant starch in the pea/maize diet in the current experiment was only 19 g/kg starch (Table 4) and in the former experiment this value was 78 g/kg starch. Furthermore, it is conceivable that it is more difficult to affect the microbial population via manipulation of the supplied substrate at considerably higher levels of bacteria in the caeca.

From the results of these two experiments it can be concluded that starch digestion rate affects broiler performance within a certain range. Diets with *in vitro* starch digestion rates (k_d) beyond 1.8 /h do not affect bird performance because slowly digestible starch levels are too low. Diets with *in vitro* starch digestion rates between 0.8 and 1.8 /h affect performance. A lower k_d results in a better FCR. Diets with *in vitro* starch digestion rates below 0.8 /h do not further improve FCR and may even increase FCR because ileal starch digestion may be reduced too much. In that circumstance, diets may contain resistant starch, which did not affect bacterial counts of *Clostridium perfringens* or *Lactobacilli* in the caeca of the birds in this experiment.

Chapter 9

General Discussion

Developments in feed evaluation

Feedstuffs and diets are generally characterised using the proximate analysis and an energy value (metabolisable energy or net energy). In the proximate analysis, moisture, crude ash, crude fat, crude protein and crude fibre are determined. The nitrogen free extract (NFE) is defined as the remaining fraction and contains starch, sugars and some other substances. Starch, the major energy supplier for monogastric animals like chickens and pigs, is neglected in the proximate analysis and in most current feed evaluation systems. Many protocols for starch analysis exist and with most protocols starch determination is not very precise. Recently, a lot of effort has been put into the development of a new protocol for the starch analysis that is suitable for all feedstuffs and diets (Brunt et al., 1998).

Most current feed evaluation systems are based on digested nutrients. In digestion trials, nutrients, as determined by the proximate analysis, are determined in the diet as well as in the faeces. Digestibility coefficients are calculated from the difference between intake and faecal output. These digestibility coefficients have been tabulated. Metabolisable energy values of feed ingredients for broiler diets are estimated from either tabulated or analysed contents of crude protein, crude fat and nitrogen free extract and tabulated digestion coefficients for these components. These nutrients are ill-defined. Crude protein consists of true protein (long chains of amino acids), peptides, amino acids and non protein nitrogen. To express the protein value, current feed evaluation systems use digestible amino acids like digestible lysine and digestible methionine instead of digestible protein. Crude fat can be divided into mono, di and triglycerides, free fatty acids, sterols, etc. The fatty acids vary in chain length and level of saturation. The starch proportion in the nitrogen free extract can vary as can the other components in this fraction. In the proximate analysis, fibre is determined as crude fibre. Neutral detergent fibre (NDF) and non starch polysaccharides (NSP) are other terms referring to the fibre fraction in feedstuffs. These fractions are defined by the protocol that is used. However, crude fibre or NDF is not fibre, but it represents fibre. Fibre can be divided into cellulose, hemicellulose, pectins and lignin.

So, it can be concluded that 'protein ain't protein', 'fat ain't fat', 'NFE ain't NFE' and 'fibre ain't fibre'. Future feed evaluation systems will be nutrient oriented. In these systems, the nutrients will be divided into glucogenic, ketogenic and aminogenic nutrients. Starch will have a more prominent position in these systems because it represents the major part of energy delivering components in poultry and pig diets and it yields only glucose. Therefore, starch is the most important glucogenic nutrient. The growth experiments described in this thesis point out that diets with similar levels of digestible starch, but different rates of starch digestion, can result in differences in feed conversion ratio (FCR) for broiler chickens. The rate of starch digestion is determined by physical factors related to the feedstuff, like particle size and botanical structures, but also by the composition and structure of the starch itself. Therefore, it can also be concluded that 'starch ain't starch'. This implies that it is not always correct to consider starches from different origin as the same and as interchangeable. In short, feed evaluation systems have evolved from nutrient composition to digestible nutrient composition and crude components have been unravelled to their underlying nutrients.

Starch digestion rate and its consequences for broiler chickens

Starch is a concentrated and very pure product. In intact seeds or roots, starch is packed in dense granules which contain amylose and amylopectin. These two polysaccharides are composed of glucose molecules only. When exposed to digestive enzymes, the size of the granules, the ratio of amylose to amylopectin and the amylopectin structure determine the susceptibility to enzymatic attack. However, the exposure of starch granules to enzymatic attack depends on the physical and chemical structures in which the granules are embedded. When intact starch sources are processed, susceptibility to enzymatic attack is generally increased substantially due to particle size reduction, opening up of the physical structures and starch gelatinisation. Therefore, starch digestion rate (proportion of starch digested per time unit) varies considerably and can be manipulated. Feeding diets with different starch digestion rate may have several effects that influence performance of broiler chickens.

Results of experiments

In this project, site and extent of starch digestion in broiler chickens were investigated for different untreated feedstuffs. Significant differences in passage rate through the small intestine were observed. These differences were related to dietary factors. Therefore, starch digestion coefficients at specific gut segments were related to mean retention times of digesta in these segments. From these factors, rate of starch digestion could be estimated. This experiment gave an indication of the differences in starch digestion rate between various feedstuffs.

It should be borne in mind that the observed digestion data only apply to those specific batches of feedstuffs. In order to be able to investigate starch digestion rate in a large number of processed and unprocessed feedstuffs, an *in vitro* method was developed. This *in vitro* method simulates the digestive process in the alimentary tract of broiler chickens. *In vitro* methods are easier, faster, less costly and more standardised than digestion experiments with live animals. On the other hand, *in vitro* systems never simulate each aspect that affects digestion. Viscosity and passage rate are examples of dietary effects that cannot easily be simulated with simple *in vitro* systems. The *in vitro* method can be used to study the variation in starch digestion rate between different batches of the same feedstuff. Another application for the *in vitro* method is to investigate the effect of different processing techniques on starch digestion rate. In a laboratory experiment the effect of different processing techniques on *in vitro* starch digestion rate was studied. The Table shows the results.

In vitro starch digestion rate (/h) of six feedstuffs as affected by various hydrothermal treatments.

	Peas	Maize	Barley	Wheat	Oats	Tapioca
Control	0.61[a]	0.77[a]	1.01[a]	1.22[a]	1.88[a]	1.79[a]
SP-60	1.17[b]	1.37[b]	1.48[b]	1.60[b]	2.34[b]	2.10[b]
EP-80/100	-	1.56[c]	1.87[c]	1.98[c]	2.49[b]	2.15[b]
EP-60/130	1.43[c]	1.88[e]	2.02[cd]	2.05[cd]	2.51[b]	2.10[b]
EP-80/130	-	1.91[ef]	2.28[ef]	2.00[c]	2.51[b]	2.10[b]
EE-100/130	2.29[d]	1.87[e]	1.89[c]	2.16[e]	2.71[c]	2.13[b]
EP-100/130	2.39[e]	2.07[g]	2.37[f]	2.15[de]	2.90[d]	2.16[b]
AT-100	1.43[c]	2.03[fg]	2.15[de]	2.13[de]	2.97[d]	2.12[b]
PT-130	2.30[de]	1.73[d]	2.22[ef]	2.29[f]	2.46[b]	-
LSD	0.10	0.13	0.19	0.11	0.19	0.22

Control = roller milled feed; SP = steam pelleting (T_{mixer}); EP = expander pelleting ($T_{mixer}/T_{expander}$); EE = expander treatment with extruderhead ($T_{mixer}/T_{expander}$); AT = toasting at atmospheric pressure (100°C; 60 min); PT =pressure toasting at 2,8 bar (130°C; 3 min).
Values within columns with the same superscript do not differ ($P > 0.05$).

These results show that hydrothermal treatments increase *in vitro* starch digestion rate of feedstuffs to a varying extent. None of the tested heat treatments left starch digestion rate unaffected in any feedstuff. The increase in starch digestion rate is caused by starch gelatinisation. Atmospheric toasting resulted in a similar starch digestion rate as expander pelleting (100/130) for maize, wheat, oats and tapioca. However, this expander treatment resulted in a significantly higher starch digestion rate of peas and barley compared to atmospheric toasting. These results clearly show that the effect of processing on starch digestion rate is feedstuff dependent.

After the *in vivo* and *in vitro* digestion experiments, four independent growth experiments have been carried out in which broiler chickens were fed diets with either slowly- or rapidly digestible starch. In the first growth experiment with broiler chickens starch sources from the same batch as used in the digestion trial were used. Based on the known starch digestion coefficients of these feedstuffs two broiler diets were formulated. These diets had the same digestible starch content, but differed in the amount of starch digested halfway the small intestine. AME- and digestible amino acid contents were similar in these diets. These two diets were used to test the hypothesis that differences in site of starch digestion affect performance of broiler chickens. This experiment revealed that broiler chickens performed better on diets in which a considerable starch fraction was digested in the posterior ileum. This probably means that a more or less continuous starch digestion over the full length of the small intestine is beneficial for growth efficiency.

The three following experiments confirmed this observation. Based on *in vitro* measurements in individual starch rich feedstuffs, diets with different starch digestion rates were formulated. In these experiments feed efficiency was better for broiler chickens fed diets with slowly digestible starch than for broiler chickens fed diets with rapidly digestible starch. In most experiments differences in starch digestion rate were established by using different types of starch rich feedstuffs. The contrast in starch digestion rate was established with peas as a source for slowly digestible starch and tapioca pellets as a source for rapidly digestible starch. In two experiments starch digestion rate was enhanced by treating the starch sources with intensive processing techniques (expander treatment and Presco expansion). In one experiment, a diet was formulated with sorghum as the only source of slowly digestible starch. Diets containing slowly digestible starch always resulted in a better feed efficiency than diets containing rapidly digestible starch, irrespective of the starch source. When differences in starch digestion rate were established by using different starch sources, the diets did also differ with regard to other dietary components. When processing was used as a method to obtain a difference in starch digestion rate, diets had exactly the same composition regarding feed ingredients. However, processing does not only alter starch structure, but may also affect the availability of lysine (Hodgkinson and Moughan, 2000), increase the soluble NSP fraction or affect other dietary components. These unavoidable side effects make it difficult to test the effect of starch digestion rate on broiler performance without any confounding factors. It can be argued to use isolated pea- and tapioca starch in experimental diets, but starch digestion rate of these isolated starches will be higher compared to the situation that the starch is still embedded within its natural structures. Furthermore, we wanted to work with starch digestion rates that can also be expected for practical broiler diets.

In one experiment (Chapter 6) FCR of birds on a diet containing slowly digestible starch (native peas and maize) was similar to that of birds on a diet with rapidly digestible starch (expander pelleted peas and maize). A diet with an intermediate starch digestion rate (steam pelleted peas and maize) resulted in a better FCR. The diets in this experiment were supplied as a mash in order to maximise the contrast in starch digestion rate. Starch digestion rate was 0.84, 1.26 and 2.39 /h for diets with native, steam pelleted and expander pelleted peas and maize respectively. The FCR of birds on these diets was 1.77, 1.73 and 1.76 respectively. Based on the differences in starch digestion rate, these results suggest that starch digestion rate of the native pea/maize diet was too slow. When starch digestion rate is too slow, total starch digestion may be impaired. This was confirmed by *in vivo* as well as *in vitro* starch digestion measurements. Starch digestion rate of the steam pelleted pea/maize diet was higher and this resulted in a higher predicted ileal starch digestion. It is most likely that this improved the FCR of the birds on this diet. Starch digestion rate of the expander pelleted pea/maize diet was very high and corresponded with a high ileal starch digestion. However, FCR of birds on this diet was significantly higher than that of the birds on the steam pelleted pea/maize diet. The *in vivo* and *in vitro* starch digestion coefficients prove that diets not only differed in starch digestion rate, but also in total extent of starch digestion. A slow starch digestion may result in incomplete starch digestion. In this circumstance, the positive effect of slowly digestible starch must at least compensate for the negative effect of a lower extent of starch digestion. In the case of the diet with native peas and maize, slowly digestible starch could not compensate for the reduced starch digestion. The diet containing steam pelleted peas and maize contained sufficient slowly digestible starch and total starch digestion was not impaired too much. The diet with expander pelleted peas and maize resulted in the highest extent of starch digestion, but did not contain slowly digestible starch and therefore FCR was higher than that of the birds with steam pelleted peas and maize.

The higher FCR of birds on native compared to steam pelleted pea/maize diets originated in the starter phase (d 0-14). In the grower phase, the chickens were older and their alimentary tract was more developed or better adapted to starch rich diets. During this phase, no differences in FCR were observed between these two treatments. These results indicate that starch digestion rate can be too low for broiler chickens to reach a high level of digestion, especially for young broiler chickens. This implies that the correlation observed between *in vitro* starch digestion and *in vivo* starch digestion of 28 day old broiler chickens is not valid for birds in the starter phase.

With these results in mind we wondered whether starch digestion rate improves FCR of broiler chickens linearly or whether an optimal level of starch digestion rate exists. Two experiments were performed in which performance was measured from respectively d 9-30 and d 7-30. This period was chosen because variation in performance is minimal in this period. Furthermore, this period is closer to the period in which *in vivo* starch digestion was measured. Performance of birds on the diet with the lowest starch digestion rate (0.73 /h) was poorer compared to the diet with a starch digestion rate of 1.05 /h in the first part of the experimental period (d 9-18). This is in line with the results in the starter phase of the previous experiment. In the second part of the experimental period this effect was not observed. Measured over the complete experimental period, these two diets resulted in a comparable weight gain and FCR. FCR of these two diets was lower than that of the diets with higher starch digestion rates. The fact that the effect of starch digestion rate on FCR levelled off at higher starch digestion rates suggests that starch digestion rate only affects bird performance in a specific range.

It was surprising to note that a slow starch digestion resulted in better bird performance than a rapid starch digestion. The *in vivo* starch digestion trial already showed that starch digestion rate was positively correlated to total starch digestion in broiler chickens. The improved FCR of birds on diets with slowly digestible starch compared to birds on diets with rapidly digestible starch indicates that either energy or protein (or both) is used more efficiently by birds on these diets. In order to test the hypothesis that a slow starch digestion improves protein efficiency, extra protein was added to a diet with slowly digestible starch (native peas and maize) and to a diet with rapidly digestible starch (expander pelleted peas and maize). The diets without extra protein did not contain excessive amounts of the most limiting amino acids. It turned out that with extra protein, the FCR decreased for birds on the diet with rapidly digestible starch, whereas the FCR was not affected for birds on the diet with slowly digestible starch. This observation means that diets with slowly digestible starch may improve protein efficiency of broiler chickens. This conclusion was confirmed in a following experiment. In this experiment, the positive effect of increasing levels of digestible amino acids on FCR was more pronounced for birds on diets with rapidly digestible starch than for birds on diets with slowly digestible starch. However, an improved protein efficiency could not explain the total difference in FCR. The efficiency of energy utilisation my also be improved when starch is digested slowly.

The physiological explanation for the positive effect of slowly digestible starch on feed efficiency can not be derived from these experiments. Starch digestion rate affects the time at which glucose is released to the bloodstream, but it also affects the amount of glucose that is absorbed at a specific site of the small intestine.

Glucose release as a function of time

The rate of glucose absorption may affect the level of synchrony between energy and protein availability in the metabolism of growing animals. Gerrits et al. (2001) showed that feeding pigs a protein rich morning meal and a starch rich evening meal instead of two times a meal containing normal amounts of protein and starch resulted in a lower nitrogen retention. They varied the level of synchrony by feeding two different diets during the day. The level of synchronisation can also be varied within a diet by varying the rates of nutrient digestion (e.g. starch and protein). Differences in the level of synchronisation within a diet will generally be less extreme than the difference created by Gerrits et al.

In the small intestine, glucose is primarily metabolised to lactate and alanine, and only a portion is completely oxidised (Mithieux, 2001). Riesenfeld et al. (1982) observed that more than a third of the glucose absorbed during a meal was converted to lactate in the intestinal wall. This way, the peak glucose influx is buffered. Lactate from the intestinal wall and from the muscles is delivered to the liver where it can be converted to glucose again or to fatty acids in situations of excessive glucose. It is likely that more glucose is converted to lactate after a rapid starch digestion than after a slow starch digestion.

The portion of absorbed glucose that is not metabolised by the intestinal wall is released to the bloodstream. As a result, plasma glucose levels are elevated after a meal and this triggers insulin release. Insulin regulates transport and uptake of glucose and amino acids by body cells, thus lowering plasma levels of these nutrients. Glucose will be utilised directly for the necessary energy supply for processes in the body. Excess glucose will be stored as glycogen in the liver and muscles. When the glycogen stores are filled, the remaining glucose will be stored as fat. Most glucose uptake takes place in the muscles. The amino acids which are taken up by the cells are incorporated into proteins. Later in the post absorptive period, energy demands are met by catabolic processes in which the animal draws on energy reserves. Glucagon stimulates hydrolysis of muscle glycogen to glucose for local use and hydrolysis of liver glycogen to raise plasma glucose levels. Liver glycogen stores are limited, but low insulin and elevated glucagon levels promote the conversion of amino acids from muscle protein to glucose and the production of free fatty acids and glycerol from adipose cells. In this metabolic state, muscles cannot utilise blood glucose as an energy source (low insulin level). Free fatty acids are used as an energy source for muscles. This way, the brain is assured of glucose supply. Glucose supply via these subsequent anabolic and catabolic processes is less efficient than direct utilisation. A more or less continuous glucose supply from the lumen enables more direct glucose utilisation. The rate of glucose absorption is obviously reflected in plasma glucose levels. A rapid rise in the plasma glucose level results in a rapid increase of the plasma insulin level. This insulin peak falls rapidly as well and may even result in an undershoot in the plasma glucose level (Wolever, 2000). In this situation glucose metabolism is not very efficient and this insulin peak results in fat production. A gradual glucose release will result in a lower, longer lasting insulin response. In this situation, more glucose is directly used and therefore less fat production will occur. Moreover, incorporation of amino acids into protein is higher because of the prolonged insulin peak. Because glucose levels are elevated for a longer time period, less gluconeogenesis (from lactate, alanine and glycerol) in the liver may be required in this situation.

Jenkins introduced the term glycemic index (GI) in 1981 to express the rise in blood glucose level after a meal. The glycemic index is determined by the amount of digestible carbohydrates in a meal as well as the rate of glucose absorption. Our digestion trial with 12 feedstuffs showed that glucose absorption rate is highly correlated to starch digestion rate in broiler chickens. This implies that the glucose absorption process is not limiting glucose uptake by the intestinal wall. Furthermore, starch digestion rate is highly correlated to glycemic index (Englyst et al, 1996) and in turn, glycemic index correlates well with insulinemic index (Björck et al., 2000).

Site of starch digestion

After leaving the gizzard, digesta are subjected to enzymatic digestion in the small intestine. Starch in diets with a high starch digestion rate is mainly digested in the anterior part of the small intestine. Feeding diets with a low starch digestion rate shift starch digestion to the posterior part of the intestinal tract. Starch digestion rate can be so low that part of the potentially digestible starch fraction leaves the small intestine undigested. This resistant starch (RS) fraction will be subjected to microbial fermentation in the hind gut. Indeed, our data indicated that the resistant starch fraction was inversely related to rate of starch digestion (Chapter 3). Our data also indicated that starch entering the hind gut of broiler chickens was not further degraded in the hind gut since starch digestion coefficients measured in the digesta in the posterior ileum were similar to those measured in the excreta. This means that the resistant starch fraction is not utilised by broiler chickens.

The amount of glucose that is absorbed in the posterior part of the small intestine is determined by the rate of starch digestion and the passage rate of gut contents. When broiler chickens consume a diet with rapidly digestible starch, no glucose will be absorbed in the posterior part of the small intestine. A diet with slowly digestible starch will provide the posterior part of the small intestine with a certain amount of glucose. However, if the passage rate through the small intestine is considerably reduced by these type of diets, lower amounts of starch will be digested in the posterior part of the small intestine. Glucose, the endproduct of enzymatic starch digestion, is rapidly absorbed from the small intestine.

Glucose is an important energy source for the body. The gut wall is a very active organ and requires a lot of energy and nutrients for its maintenance. Intestinal transport of absorbed nutrients coincides with their partial catabolism in the gut (Vaugelade et al., 1994). Amino acids and glucose are used as fuel for the gut wall after their absorption. It is generally believed that the contribution of glucose to fulfil the energy demands of the intestinal wall is limited. Glutamine is generally considered as the major energy provider for the intestinal wall. However, glucose can influence the extent of glutamine oxidation and according to Plauth et al. (1999) glucose and glutamine may partially substitute each other to the purpose of a metabolic fuel. Fleming et al. (1997) conclude that glucose and glutamine provide similar proportions of energy to mucosal cells of the small intestine of rats. Reeds et al. (2000) state that the metabolic functions of enterocytes change following isolation procedures. According to this group, glucose oxidation in the mucosa is low under *in vivo* conditions while glucose is easily metabolised via the TCA cycle by isolated enterocytes. There are indications that the capacity of enterocytes in the posterior part of the small intestine to oxidise glucose is smaller than that of enterocytes in the anterior parts. However, glucose supply to the posterior part of the small intestine may contribute to the energy supply of the intestinal wall. All the more because a local excess of absorbed glucose in the anterior part of the small intestine does not affect the energy supply of the small intestine (Fleming et al., 1997). If part of this excess glucose is shifted to the posterior part where it can serve as an energy source, this glucose may spare amino acids from being oxidised.

When starch is completely digested in the anterior part of the small intestine, no glucose will be available in the gut wall of the posterior part of the small intestine. In this situation, the gut wall may oxidise more amino acids for its energy requirements and this may have a negative effect on protein efficiency and thus performance.

Most studies on nutrient oxidation in the intestinal wall focus on the jejunum and the colon and are performed under *in vitro* conditions. It is generally believed that the wall of the small intestine prefers glutamine as its energy source. However, under *in vivo* conditions, glucose concentrations in the lumen of the small intestine are likely to be much higher than glutamine concentrations. This would implicate that glucose oxidation is quantitatively more important than glutamine oxidation. Indeed, Kight and Fleming (1993) concluded that *in vivo* glucose oxidation by enterocytes should not be limited by the *in vivo* glucose concentration, whereas glutamine concentration is potentially limited. Glucose is primarily metabolised to lactate and alanine, and only a portion is completely oxidised (Windmueller and Spaeth, 1980; Mithieux, 2001). Glucose contributed at least 10% of the total respired carbon dioxide produced by a jejunal segment in fed rats (Windmueller and Spaeth, 1980).

Consequences of resistant starch

Digestion of feedstuffs is a combination of physical particle size reduction and enzymatic breakdown of chemical structures. Both processes take time. When digestion is slow and passage rate through the alimentary tract is rapid, it is possible that potentially digestible starch is excreted undigested. It is also possible that a certain proportion of the starch is resistant to digestion. Undigested starch is wasted for the broiler chicken.

In our digestion trial we observed that for most feedstuffs total starch digestion was the same as ileal starch digestion. This means that starch fermentation in the hind gut was neglectible. In broiler chickens, most microbes are found in the caeca. It may be that amylolytic bacteria are outnumbered by other strains in the caeca. Another explanation may be that most starch particles do not enter the caeca and are therefore not suscepted to microbial fermentation. However, in one of our experiments (Chapter 6) we observed less colony forming units of *Clostridium perfringens* bacteria after feeding a diet with slowly digestible starch than after feeding a diet with rapidly digestible starch. This may implicate that *Clostridium perfringens* grows less well in a starch rich environment or that they are suppressed by strains of microbes that thrive well in this environment. Van der Steen et al. (1997) observed that *Clostridium perfringens* grow well in a protein rich environment. In another experiment (Chapter 8) the effect of starch digestion rate on *Clostridium perfringens* counts pointed in the same direction, but this time the difference was not significant. It may be that feeding a slowly digestible starch diet increases the proportion of amylolytic bacteria at the expense of *Clostridia* and other proteolytic bacteria. This is a positive effect because protein fermentation is regarded as negative. *Clostridium perfringens* in particular is regarded as negative because this strain can produce a toxin that causes necrotic enteritis. This disease will cause damage to the gut wall which impairs performance and may lead to increased mortality rates. In situations when the gut wall is damaged digestibility of nutrients will be impaired and in those circumstances it may be better to supply diets with rapidly digestible nutrients including starch. During fermentation of carbohydrates volatile fatty acids are produced which can be utilised as energy sources for the bird. Butyric acid in particular is regarded as an important nutrient for the gut wall. However, microbial fermentation of starch yields less energy than enzymatic starch digestion in the small intestine.

Effect of slowly digestible starch on protein and energy efficiency

The explanations for the observed effects of starch digestion rate on broiler performance may be found in the physiological mechanisms described above. The observation that the effect of starch digestion rate on performance is more pronounced at low dietary amino acid levels indicate that slowly digestible starch has a protein sparing effect. However, at high dietary amino acid levels FCR was still better for birds on slowly digestible starch diets. This implies that energy is also utilised more efficiently when starch digestion is slow instead of rapid. The energy saving effect may be found in the fact that a more or less continuous glucose absorption from the small intestine leads to more direct glucose utilisation compared to a peak glucose release which has to be stored temporarily in glycogen or fat tissues. In the former situation more energy is stored in the energy carrier ATP. The protein sparing effect of slowly digestible starch may be found in a more efficient amino acid incorporation in protein as a result of a prolonged insulin release, but can also be explained by sparing amino acids from oxidation in the intestinal wall. Maybe both mechanisms are involved. The observation that beyond a certain level, starch digestion rate does not affect FCR anymore, suggests that site of starch digestion plays a more important role than the hormonal effects.

Economical consequences

When FCR is reduced with 0.01 g feed per g gain and total weight gain is 2,000 g, then 20 g less feed per bird is required to reach this weight. For a poultry farm with 30,000 broiler chickens per round and seven rounds per year, this saves 4,200 kg of feed per year. With an average cost for broiler feeds of € 0.26 per kg, this reduction in FCR results in € 1,092.00 less feed costs per year.

In 2000, ± 350 million broiler chickens were produced in The Netherlands. Twenty g less feed per animal corresponds to 7 million kg of feed, which equals ± € 1.8 million. In our experiments the difference in FCR between slowly digestible starch and rapidly digestible starch diets was at least 0.03 g feed per g gain and this may save 21 million kg feed, which equals € 5.5 million.

For a good comparison, the price difference between slowly digestible starch and rapidly digestible starch diets has to be included. Feeds containing slowly digestible starch sources like peas and sorghum are generally more expensive. However, feeds with slowly digestible starch can also be obtained when current feeds with maize and wheat as major components are less intensively pelleted. Intensive precompaction processes increase starch digestion rate considerably. Less intensive pelleting saves energy cost, however, production capacity and pellet quality are factors that must be considered as well. The protein sparing effect of slowly digestible starch can be translated to lower amino acid requirements in broiler feeds with slowly digestible starch. In addition, feeding diets with slowly digestible starch sources increases the net energy value of these feedstuffs.

Practical consequences

Manipulation of starch digestion rate can be applied by selection of starch sources or by processing of starch sources. Therefore, both nutritionists and process engineers should be aware of the importance of starch digestion rate.

Poultry nutritionist

The poultry nutritionist should be aware of the fact that starch from one feedstuff cannot always be exchanged for starch from another feedstuff ('starch ain't starch'). This may have detrimental effects on broiler performance. Starch from one feedstuff is less digestible than that from another and starch sources differ in starch digestion rate. This has consequences for insulin release and site of starch digestion in the small intestine of broiler chickens. When diets with a slow starch digestion are required, then the nutritionist has to select feedstuffs with that property (peas, beans, sorghum) and must assure that this property is not wasted by severe processing in the feed mill. In situations when the gut wall of the birds is damaged, it may be advisable to supply diets with rapidly digestible starch. In this situation other starch sources should be selected or processing conditions during pelleting should be more severe.

Process engineer

Processing techniques used in today's feed mills only increase starch digestion rate. It is not easy to reduce starch digestion rate by means of processing in the feed mill. Most broiler feeds contain considerable amounts of wheat and maize and minor amounts of peas. The feeding value of the native starch in these feedstuffs is better than that of the processed starch after the feed has been precompacted and pelleted. However, for several reasons it is preferred to feed pellets instead of mash feed. Therefore, it is the challenge for the process engineer to make a good quality pellet with a minimal effect on starch accessibility.

Further research

Processing increases starch digestion rate of feedstuffs. This means that slowly digestible starch sources can be altered in rapidly digestible starch sources. It would be interesting if starch digestion rate of rapidly digestible starch sources can be reduced somehow. In this regard, chemical treatments may have potential. Other dietary components may also have an effect on starch digestion rate. When soluble NSP are included in the diet, viscosity in the small intestine will increase. This lowers the diffusion rate of enzymes through the digesta, leading to a slower digestion. Unfortunately, effects on gut viscosity can not be measured in our *in vitro* system.

We have observed an interaction between starch digestion rate and protein level on FCR. It is likely that synchronisation of starch and protein digestion may increase feed efficiency. Therefore, it may be interesting to investigate the variation in protein digestion rate. When *in vitro* protein digestion rate can be determined as well, a growth experiment can be designed in which the effect of different levels of synchronisation of starch and protein digestion on performance can be compared. For practical implementation it is important to be able to quantify the effect of starch digestion rate on FCR. It is important to know which part is caused by a protein sparing effect and which part is caused by an energy saving effect.

We observed a reduced FCR when slowly digestible starch was fed to broiler chickens. It would be interesting to know whether starch digestion rate affects slaughter characteristics. Measuring blood parameters (glucose, insulin, lactate) may enhance our understanding of the physiological background of the observed effects.

The *in vitro* method can be used to study the variation in starch digestion rate within feedstuffs (different batches, year effects, geographical effects). Broiler feed manufacturers can use this method to investigate the effect of their mill equipment on starch digestion rate. With that knowledge they can optimise the pelleting lines in their feed mills.

This research has focussed on the effect of starch digestion rate on performance of broiler chickens. It appeared that the *in vitro* method as used in this project predicts starch digestion in 4 wk old broiler chickens well. For younger birds, predicted starch digestion was probably too high. It may be worthwhile to develop an *in vitro* system for young birds (2 wk old) and perhaps also for other animal species.

Finally, it may be interesting to study the effects of resistant starch on microbial populations in the gastrointestinal tract of farm animals. Gut health may benefit from the inclusion of a small amount of resistant starch when bacteria that are negative for gut health are partly replaced by bacteria that are positive for gut health.

Conclusions

From the experiments described in this thesis, the following conclusions can be drawn:

1. Site, rate and extent of starch digestion vary considerably among feedstuffs.
2. Hydrothermal treatments increase starch digestion rate by means of starch gelatinisation. The effect of hydrothermal treatments on starch digestion rate are feedstuff dependent.
3. For slowly digestible starch sources, ileal starch digestion is related to rate of starch digestion.

4. Our *in vitro* system, which simulates the digestive process in the broiler alimentary tract, gives a good prediction of rate and extent of starch digestion in broiler chickens.
5. Diets containing slowly digestible starch result in better broiler performance than diets containing rapidly digestible starch. Slowly digestible starch improves protein- and energy efficiency in broiler chickens.
6. Feeding resistant starch reduces the number of *Clostridium perfringens* bacteria in the caeca of broiler chickens.

Summary

In feed evaluation most attention is given to the energy- and protein value of a feedstuff. Energy and protein are necessary for maintenance and production. Energy is not a chemically identifiable nutrient but is stored in organic compounds like carbohydrates, proteins and fats. Energy is a property that is manifested when these compounds are oxidised during metabolism. A balanced diet provides the birds with sufficient energy and sufficient building blocks.

Apparent metabolisable energy (AME) and digestible amino acids are the most prominent constituents in current broiler feed evaluation systems. In The Netherlands, AME is derived from digestible crude protein, crude fat and nitrogen free extract (NFE). More than 50% of the daily energy intake is provided by starch which is included in the NFE fraction.

Starch is a storage carbohydrate in plants which can be found in granules of varying size. It is composed of two glucose polymers: amylose and amylopectin. Amylopectin, which is the major component of starch, is a branched molecule and gives the starch granule a certain level of crystallinity. The molecular structure of starch determines its resistance to enzymatic degradation. This molecular structure can be modified by treatments in which heat and moisture are involved. During these treatments the crystalline structure disappears and an amorphous structure prevails. This process is referred to as starch gelatinisation. Gelatinised starch is more prone to enzymatic digestion because the accessibility for enzymes has increased dramatically. However, starch digestion is not only determined by the properties of the starch fraction itself. Accessibility is also determined by the cell wall- and protein structures surrounding the starch granules. These structures within the feedstuff can protect starch granules from digestive enzymes. When feedstuffs are broken into smaller particles by milling, then the protective effect of these structures are reduced and the surface area per gram increases. Therefore, particle size reduction increases accessibility in two different ways.

A digestion trial pointed out that starch digestion of various native feedstuffs is not complete in four week old broiler chickens. Differences were observed in site, rate and extent of starch digestion. The major part of starch was digested in the anterior part of the small intestine and differences were most pronounced in this part. Legumes like peas and beans contained a considerable starch fraction that was digested in the second half of the small intestine. Total starch digestion was not limited by the retention time in the gastrointestinal tract for most feedstuffs. However, for feedstuffs with a slow starch digestion, like legumes and potato starch, retention time appeared to limit total extent of starch digestion. Microbial fermentation of starch that was not digested in the small intestine did not occur in the hind gut, as total starch digestion was similar to ileal starch digestion.

An *in vitro* method that simulates the digestive process in the broiler alimentary tract was developed. *In vitro* starch digestion correlated well with site, rate and extent of *in vivo* starch digestion and *in vitro* starch digestion curves were found to be additive. Based on *in vitro* measurements starch could be fractionated in rapidly digestible starch (RDS_p), slowly digestible starch (SDS_p) and resistant starch (RS_p). Starch digestion curves of legumes and waxy maize were better fitted in a two-phase model than a one-phase model. This suggests that two distinct starch fractions are digested at a different rate. This was also noticed in compound feeds containing more than one starch source. Both *in vivo* and *in vitro* measurements indicated that there are major differences in starch digestion rate between feedstuffs. However, the practical relevance of starch digestion rate in broiler nutrition was unknown.

In a first growth experiment, starch sources from the same batches as used in the digestion trial were used to formulate diets with similar contents of digestible starch but different amounts of SDS. In this experiment broiler chickens consumed more feed and grew faster and more efficient on diets containing a high amount of SDS compared to broiler chickens on diets with a low amount of SDS.

Because treatments in this experiment differed considerably in dietary composition another experiment was performed in which contrasts in starch digestion rate were established in two ways. Again two diets were formulated with either peas (slow) and maize (gradual) or tapioca (rapid) and wheat (gradual). The starch sources were split in three sub batches. One sub batch was left untreated, one was steam pelleted and one was expander pelleted. These technological treatments increased starch digestion rate of the starch sources. Starch digestion rate of diets with steam pelleted starch sources was higher than that of untreated starch sources and starch digestion rate of expander pelleted starch sources was highest. The pellets were coarsely milled before mixing with the other dietary components. The starch sources from each sub batch were included in six experimental diets: native pea/maize, steam pelleted pea/maize, expander pelleted pea/maize, native tapioca/wheat, steam pelleted tapioca/wheat and expander pelleted tapioca/wheat. Due to differences in particle size distribution, no clear statements could be made regarding feed intake and weight gain. However, the positive effect of a slow starch digestion on feed efficiency was confirmed in this experiment. Feed efficiency was better for broiler chickens on diets with native peas and maize compared to that of birds on diets with either native tapioca and wheat or with expander treated peas and maize. In both cases starch digestion rate was substantially higher than for native peas and maize. Furthermore, with regard to feed efficiency, an interaction was observed between starch digestion rate and amino acid level. Feed efficiency improved when extra amino acids were added to a diet with rapidly digestible starch, but not when they were added to a diet with slowly digestible starch.

In the following experiment five levels of amino acids were incorporated in diets with either slowly digestible starch (a pea/maize diet) or rapidly digestible starch (a tapioca/maize diet). Across all amino acid levels weight gain was higher and feed conversion ratio (FCR) was lower for birds on pea/maize diets than for birds on tapioca/maize diets. The difference in weight gain and FCR was most pronounced at the lowest level of amino acids. This interaction between starch digestion rate and amino acid level is in line with the results in the previous experiment.

The positive effect of slowly digestible starch on feed efficiency and the observation that the effect was most pronounced at low amino acid levels suggest that protein efficiency is higher when slowly digestible starch is fed as compared to rapidly digestible starch. A slow starch digestion results in more or less continuous glucose release to the bloodstream and as a result insulin is also gradually released. Insulin regulates transport and uptake of amino acids by body cells. When amino acid supply is sufficient, a prolonged period of elevated plasma insulin level results in more incorporation of amino acids into protein. Another consequence of a slow starch digestion is that part of the starch is digested in the posterior part of the small intestine. Oxidation of glucose that is absorbed in this part of the small intestine fuels the metabolism of the intestinal wall. Other fuels for the intestinal wall are amino acids like glutamine. When glucose is not available in this part of the small intestine, additional amino acids may be oxidised to meet the energy demands of the gut wall. This may occur when a diet containing rapidly digestible starch is fed to broiler chickens. It is conceivable that more glucose is directly used when it is gradually released from the small intestine compared to a rapid release. After a rapid glucose release excessive glucose is stored as glycogen and fat. In periods of glucose deficiency glycogen can be converted to glucose again and fat can be oxidised to yield energy. Energy is lost during these conversions. Therefore, feeding slowly digestible starch instead of rapidly digestible starch can also increase energy efficiency.

Results of two experiments in which the response of broiler chickens to varying levels of starch digestion rate was investigated suggest that performance of broiler chickens is affected by starch digestion rate within a certain range of starch digestion rate. The FCR of broiler chickens respond to increasing levels of starch digestion rate according to a sigmoid curve. Beyond a certain level of starch digestion rate, the amount of slowly digestible starch is too low to have a positive effect on FCR. Within a certain range of starch digestion rate, FCR improves when the rate increases. And below a threshold value of starch digestion rate, FCR is not affected or may even increase due to impaired ileal starch digestion. These low starch digestion rates are not expected to be found in practical conditions because most broiler diets are pelleted and the feedstuffs that are used in commercial broiler diets have higher starch digestion rates.

From the results of the four growth experiments it can be concluded that starch digestion rate has a practical relevance in broiler nutrition. This means that the poultry nutritionist should be aware of the differences in starch digestion rate between feedstuffs. Starch digestion rate can be increased, but not decreased by processing in the feed mill. Therefore, the process engineer should also be aware of the consequences of preconditioning and pelleting conditions on starch digestion rate. The *in vitro* method is an effective tool to evaluate the effect of the existing pelleting line on starch digestion rate.

Digestion coefficients were determined in four week old broiler chickens. It is known

that digestive capacity is not optimal yet in the young chick. The digestive system may still be developing or the bird may still be adapting to the starch rich diets it is fed. Contradicting effects of starch digestion rate on performance of young birds may be explained by the fact that their starch digestion capacity is not optimal yet. Therefore, another *in vitro* protocol is required to predict starch digestion in these young animals.

A positive correlation between slowly digestible starch and resistant starch was observed in the digestion trial. This means that when diets with slowly digestible starch are fed to broiler chickens, a fraction of starch may leave the small intestine undigested. This may cause a shift in microbial populations from protein and fibre degrading bacteria to starch degrading bacteria. In one experiment we observed less colony forming units of *Clostridium perfringens* in caecal contents of birds fed a slowly digestible starch diet than in that of birds fed a rapidly digestible starch diet. *Clostridium perfringens* are protein degrading bacteria which can produce a toxin that causes necrotic enteritis. Therefore, feeding diets containing small amounts of resistant starch may be beneficial for a good gut health.

Samenvatting

Behoefte aan energie en bouwstoffen

Ieder levend wezen heeft een dagelijkse behoefte aan brandstoffen (energie) en bouwstoffen om in leven te blijven. Zo is er een energiebehoefte voor het instandhouden van de complexe toestand van het lichaam en ter ondersteuning van het normaal functioneren van het lichaam: bloedsomloop, ademhaling, zenuwstelsel, spiertonus, vertering, hersenactiviteit, etc. Dit wordt de basale energiebehoefte genoemd. Ook voor de activiteit en de warmteregulatie van het individu is energie nodig. Samen met de basale energiebehoefte vormt dit de onderhoudsbehoefte. Mensen die dagelijks een extreme inspanning leveren zoals topsporters en in mindere mate mensen die voor hun werk zware arbeid verrichten, hebben een grotere energiebehoefte dan de gemiddelde mens. Deze extra behoefte wordt de energiebehoefte voor productie genoemd. Bij landbouwhuisdieren is extra energie nodig voor groei (vleesaanzet) of productie van eieren of melk. Een hoogproductief dier (melkkoe of vleeskuiken) kun je dus vergelijken met een topsporter. De vleeskuikens die in Nederland gehouden worden hebben een erfelijke aanleg om in korte tijd zeer snel te groeien. Na ongeveer zes weken zijn ze slachtrijp. De gewichtstoename bestaat voornamelijk uit eiwit, oftewel vlees.

Aanbod van energie en bouwstoffen

De dagelijkse voeding van landbouwhuisdieren is erop gericht om in de dagelijkse behoefte van de dieren te voorzien. De belangrijkste componenten in de hedendaagse veevoeding zijn energie en eiwit. Daarnaast worden er ook eisen gesteld aan de dagelijkse mineralen- en vitaminevoorziening, maar dan praten we over veel kleinere hoeveelheden. Water en zuurstof zijn ook essentiële stoffen, maar die worden buiten beschouwing gelaten omdat deze stoffen via andere wegen dan de voeding opgenomen worden. De energie in voedermiddelen is via de fotosynthese (zonne-energie) vastgelegd in organische verbindingen. Als zodanig is energie dus niet een chemisch identificeerbare voedingsstof. Bij verbranding van voedingsstoffen als koolhydraten, aminozuren en vetten tijdens de stofwisseling komt de vastgelegde energie weer vrij. Niet alle energie in een voeder komt het dier daadwerkelijk ten goede. Een deel van de energierijke verbindingen wordt niet verteerd in het maagdarmkanaal en wordt met de mest weer uitgescheiden. Een ander deel wordt wel verteerd, maar wordt niet benut en verlaat het dier via de urine of via gassen als waterstof, kooldioxide en methaan. Dan is er nog een deel van de energie dat omgezet wordt in warmte, wat deels wel benut wordt voor de regulatie van de lichaamstemperatuur. Het resterende deel wordt benut voor het onderhoud van het lichaam en de productie. In Nederland wordt de hoeveelheid omzetbare energie in vleeskuikenvoeders geschat op basis van de gehaltes aan verteerbaar eiwit, verteerbaar vet en verteerbare overige koolhydraten (waarvan een groot deel uit zetmeel bestaat). Organische verbindingen zoals eiwitten, vetten en koolhydraten kunnen, nadat ze in het maagdarmkanaal tot kleinere brokstukken (aminozuren, peptiden, glycerol, vetzuren, glucose, etc.) verteerd zijn, opgenomen worden door het lichaam. Afhankelijk van de situatie in het lichaam en de balans van de voeding worden deze verbindingen vervolgens gebruikt als bouwstof voor eiwitten (spieren, enzymen, etc.), vetten of glycogeen (energiereserve) of ze worden verbrand om in de energiebehoefte van het lichaam te voorzien.

Zetmeel, de hofleverancier voor energie

Dit proefschrift is op zetmeel gericht. Meer dan de helft van de beschikbare energie in vleeskuikenvoeders wordt door zetmeel geleverd. Zetmeel is een koolhydraatverbinding die als energieopslag dient voor planten. Zetmeel bestaat uit twee typen glucoseverbindingen: amylose en amylopectine. Deze moleculen zijn stevig samengepakt in zogenaamde zetmeelgranules die te vinden zijn in de zaden van granen en peulen, maar ook in aardappels en tapiocawortelen. De precieze samenstelling en structuur van zetmeel verschilt aanzienlijk tussen diverse plantensoorten en de structuur kan door blootstelling aan processen met hoge temperaturen en voldoende water ook nog eens behoorlijk veranderen. Zetmeel wordt niet als zodanig gevoerd, maar maakt onderdeel uit van een complexe structuur van bijvoorbeeld een graankorrel. Het zetmeel kan alleen door het dier benut worden als de verteringsenzymen bij het zetmeel kunnen komen en het vervolgens kunnen afbreken tot glucose. Het glucose wordt vervolgens door de darmwand opgenomen (geabsorbeerd) en kan dienst doen als energiebron of als bouwstof. De toegankelijkheid van zetmeel voor enzymen wordt vergroot door voedermiddelen te malen in de mengvoederfabriek. Bepaalde celwandstructuren kunnen echter ook na het maalproces de toegankelijkheid van het zetmeel nog beperken. Al deze factoren maken dat er grote verschillen zijn in de verteerbaarheid van het zetmeel. De toegankelijkheid van zetmeel is de beperkende factor in de zetmeelvertering. Aangenomen wordt dat er in het darmkanaal een overmaat aan zetmeelsplitsende enzymen is. Bij een gemakkelijk verteerbare zetmeelbron zal praktisch al het zetmeel in het voorste gedeelte van de dunne darm, de twaalfvingerige darm, verteerd en geabsorbeerd worden. De vertering van een voeder waarvan het zetmeel moeilijker toegankelijk en moeilijker afbreekbaar is zal dus meer tijd in beslag nemen. En doordat de voederbrij met een bepaalde snelheid door het darmkanaal stroomt zal het glucose later en verder achterin de dunne darm pas geabsorbeerd worden. Er zijn ook grondstoffen waarvan het zetmeel zo slecht verteert dat een deel onverteerd de dunne darm verlaat. Dit deel wordt resistent zetmeel genoemd en zal vervolgens worden blootgesteld aan microbiële fermentatie in de dikke darm of in de blinde darmen. Als het zetmeel ook door de bacteriën niet afgebroken wordt, dan zal het onbenut het dier weer met de mest verlaten.

Het ene zetmeel is het andere niet

In de voederwaardering voor vleeskuikens wordt momenteel geen onderscheid gemaakt tussen zetmeel van diverse afkomst. Sterker nog, zetmeel is niet eens als zodanig opgenomen in de schattingsformule voor de omzetbare energie van vleeskuikenvoeders. De gehaltes van een aantal voedingsstoffen worden geanalyseerd en de restfractie wordt overige koolhydraten genoemd. Deze fractie is dus zeer afhankelijk van de nauwkeurigheid en betrouwbaarheid van de analyses van de andere voedingsstoffen. In zetmeelrijke grondstoffen of voeders is zetmeel de belangrijkste component van deze restfractie. Op basis van proeven die in de internationale vakliteratuur zijn beschreven, blijkt dat er verschillen bestaan in de mate van zetmeelvertering in vleeskuikens. Deze verschillen worden deels door verschillen in zetmeelsamenstelling en -structuur veroorzaakt. Echter, de invloed van beschermende structuren, zoals moeilijk doordringbare celwanden, kan ook enorm zijn. Tenslotte worden grondstoffen voordat ze in het uiteindelijke vleeskuikenvoer belanden vaak eerst gemalen en later samen met de andere voercomponenten onder stoomtoevoeging in een korreltje geperst. Hierbij worden nogal eens temperaturen bereikt die de zetmeelstructuur zodanig veranderen dat de verteerbaarheid enorm toeneemt. In een eerste verteringsproef hebben wij de vertering van zetmeel uit een twaalftal grondstoffen onderzocht in vleeskuikens van vier weken oud. Het betrof hier voornamelijk grondstoffen die niet aan enige warmtebehandeling hadden blootgestaan. De grondstoffen waren echter wel gemalen met een hamermolen over een zeef van 2,75 mm. Er was hier dus sprake van de vertering van natief zetmeel, waarbij het wel mogelijk was dat zetmeelgranules tijdens het malen in meer of mindere mate beschadigd waren. Deze proef toonde een aantal zaken aan. Ten eerste waren er verschillen in de mate van zetmeelvertering. Voor de meeste grondstoffen was de mate van zetmeelvertering hoog. Maar de zetmeelvertering van een aantal grondstoffen zoals aardappelzetmeel en in mindere mate erwten en bonen, was matig te noemen. De onverteerde zetmeelfractie varieerde van 1% voor tapioca tot 69% voor natief aardappelzetmeel. Ten tweede bleek dat er aanzienlijke verschillen waren in de snelheid van de zetmeelvertering. Een hogere verteringssnelheid bij eenzelfde passagesnelheid door de dunne darm heeft tot consequentie dat er meer zetmeel voorin de dunne darm wordt verteerd en dus minder achterin de dunne darm. Voor alle onderzochte grondstoffen gold dat het grootste deel van het verteerde zetmeel in de voorste helft van de dunne darm werd verteerd. Tenslotte zagen we dat de verteringscoëfficiënt van zetmeel na passage door de dunne darm even hoog was als na passage door het hele maagdarmkanaal. Dit betekent dat er geen noemenswaardige microbiële fermentatie van zetmeel heeft plaatsgevonden in de dikke darm of in de blinde darmen.

Simulatie van de zetmeelvertering in het laboratorium

Omdat zowel de mate als de snelheid van de zetmeelvertering in vleeskuikens verschilt per grondstof en bovendien beïnvloedt kan worden door technologische behandelingen is er behoefte om deze eigenschappen routinematig te kunnen meten in een voedermiddel. Het is natuurlijk niet de bedoeling om hier elke keer kuikens voor te gebruiken. Vandaar dat we gewerkt hebben aan de ontwikkeling van een *in vitro* methode die de verteringsprocessen in het maagdarmkanaal van vleeskuikens nabootst. Een monster van een voedermiddel wordt eerst gemalen om de maalwerking van de spiermaag te simuleren. Vervolgens wordt het monster in een reageerbuis in twee stappen blootgesteld aan enzymmengsels die de passage door de kliermaag en de dunne darm simuleren. Op gezette tijden wordt het vrijgekomen glucose gemeten en hieruit kan de hoeveelheid verteerd zetmeel berekend worden. Door de zetmeelvertering tegen de incubatietijd uit te zetten kan de verteringssnelheid geschat worden. Op deze manier hebben we relaties kunnen leggen tussen de *in vitro* zetmeelvertering na 2 en 4 uur en de zetmeelvertering halverwege en aan het einde van de dunne darm van het vleeskuiken. Bovendien bleek er een goede relatie te zijn tussen de *in vitro* verteringssnelheid van zetmeel en de verteringssnelheid van zetmeel gemeten in het dier. Doordat we nu een meetinstrument in handen hadden die de vertering van zetmeel uit verschillende grondstoffen in het vleeskuiken kan voorspellen werd het mogelijk grondstoffen hierop te selecteren. Met deze *in vitro* methode konden we het zetmeel in voeders onderverdelen in fracties snel verteerbaar zetmeel (SVZ), langzaam verteerbaar zetmeel (LVZ) en resistent zetmeel (RZ).

De plaats van zetmeelvertering in de dunne darm

Van de grondstoffen die we in de verteringsproef hebben onderzocht hebben we een deel bewaard voor een groeiproef met vleeskuikens. Met behulp van de verteringscijfers van deze grondstoffen konden we twee vleeskuikenvoeders formuleren die dezelfde hoeveelheid verteerbaar zetmeel bevatten, maar waarvan in het ene voeder 99% en in het andere voeder slechts 85% van het verteerbare zetmeel in de eerste helft van de dunne darm verteert. Dit houdt dus in dat 1 respectievelijk 15% van het verteerbare zetmeel in de tweede helft van de dunne darm verteert. Verder bevatten beide voeders evenveel beschikbare energie en verteerbare aminozuren. We wilden onderzoeken of vleeskuikens even snel en efficiënt groeien op voeders waarvan de plaats van zetmeelvertering verschilt. De kuikens op het langzaam verteerbare voer (15% achterin) namen meer voer op en groeiden sneller en efficiënter dan de kuikens op het snel verteerbare voer (1% achterin). Een efficiëntere groei wil zeggen dat de kuikens minder voer nodig hebben om een gram te groeien. Dit wordt uitgedrukt in voederconversie (gram voeropname per gram groei). Dieren met een lage voederconversie zijn dus efficiënter omgegaan met de opgenomen voedingsstoffen dan dieren met een hoge voederconversie.

De snelheid van zetmeelvertering in de dunne darm

De resultaten van de eerste groeiproef waren verrassend. Voor zover wij weten is dit effect nog niet eerder beschreven. Doordat we echter een verschil in zetmeeleigenschappen creëerden door verschillende zetmeelhoudende grondstoffen in de beide proefvoeders op te nemen zagen de proefvoeders er qua samenstelling wel heel verschillend uit. Dit is niet echt gewenst omdat naast de gewenste verschillen ook andere verschillen tussen de proefvoeders aanwezig kunnen zijn. Zo kunnen bepaalde grondstoffen specifieke eigenschappen hebben waarvan wij ons niet bewust zijn, terwijl andere grondstoffen deze eigenschappen niet hebben. Op die manier kunnen de zetmeeleigenschappen dus verstrengeld zijn met andere eigenschappen. Om wat meer zekerheid te krijgen dat het gevonden positieve effect van langzaam verteerbaar zetmeel op de technische resultaten van vleeskuikens ook daadwerkelijk door verschillen in zetmeel veroorzaakt is, hebben we een tweede groeiproef opgezet. In deze proef hebben we op twee manieren een verschil in snelheid van zetmeelvertering gecreëerd. We hebben een voer met langzaam verteerbaar zetmeel geformuleerd met erwten en mais als zetmeelbronnen en een voer met snel verteerbaar zetmeel met tapioca en tarwe als zetmeelbronnen. Een deel van de zetmeelbronnen hebben we alleen gemalen, een ander deel na malen onder stoomtoevoeging mild geperst bij ± 60°C en een derde deel hebben we na malen onder stoomtoevoeging intensief geperst (na voorverdichting in een zogenaamde expander) bij ± 130°C. De brokken die het resultaat zijn van de persbehandelingen zijn vervolgens weer (grof) gemalen om structuurverschillen te voorkomen. Deze persbehandelingen leidden tot een zekere mate van zetmeelverstijfseling. Dit is een proces waarbij de structuur van het zetmeel zodanig wordt veranderd, dat het zetmeel gemakkelijker door verteringsenzymen afgebroken wordt. Het gevolg was dat er door deze technologische behandelingen ook een contrast in de snelheid van zetmeelvertering is aangebracht binnen dezelfde grondstoffen (zie Tabel).

Proefopzet waarbij op twee manieren (zetmeelbronnen en technologie) een contrast in de snelheid van zetmeelvertering werd aangelegd.

Zetmeelbron	Snelheid van *in vitro* zetmeelvertering (fractie per uur)		
	Natief	Geperst (± 60°C)	Geperst (± 130°C)
Erwten / mais	0,8	1,3	2,4
Tapioca / tarwe	2,0	2,5	3,0

Dit heeft als voordeel dat de grondstofsamenstelling van de proefvoeders gelijk was. Op deze manier konden we het effect van zetmeel onderzoeken zonder dat dit effect verstrengeld was met een andere voersamenstelling. Er dient wel aangetekend te worden dat door deze technologische behandelingen ook andere dan zetmeeleigenschappen veranderd kunnen worden. Dit betreft een schoolvoorbeeld van de wet van behoud van ellende.

De vleeskuikens die het voer met de mild geperste erwten en mais kregen lieten de laagste voederconversie zien (1,73). De kuikens op de andere voeders hadden een hogere voederconversie. Op basis van de vorige proef verwachtten we dat de kuikens op het voer met natieve (onverhitte) erwten en mais de laagste voederconversie zouden halen. Deze voeders hadden immers de traagste *in vitro* zetmeelvertering. De waarneming dat de kuikens op het mild geperste erwten/mais voer het beter deden dan die op het intensief geperste erwten/mais voer en die op de tapioca/tarwe voeders was op basis van de *in vitro* zetmeelvertering te verwachten. Bij nadere beschouwing bleek dat de kuikens op het natieve erwten/mais voer in het begintraject (van 0 tot 14 dagen leeftijd) met name een slechte voerbenutting lieten zien. In dit leeftijdstraject is de verteringscapaciteit van de kuikens nog niet optimaal en moeten de dieren nog wennen aan de voeders. In die situatie hebben de kuikens met name moeite met de vertering van slecht toegankelijke voedingsstoffen zoals het zetmeel in dit specifieke voer. Dus uiteindelijk waren de resultaten van deze proef toch te verklaren. En omdat beide contrasten in snelheid van zetmeelvertering (door technologie en door grondstofsamenstelling) tot dezelfde conclusie leidden waren we meer overtuigd van het feit dat het hier een echt effect van de snelheid van zetmeelvertering betreft.

Langzaam verteerbaar zetmeel en eiwitbenutting

In de hierboven beschreven proef zijn nog twee extra voeders gemaakt die in grote lijnen overeenkwamen met de voeders van het natieve erwten/mais voer (langzaam) en die van het intensief geperste erwten/mais voer (snel). Aan deze extra voeders is echter wat extra eiwit toegevoegd. Nu bleek dat de toevoeging van extra eiwit geen effect had op de voederconversie van de kuikens op het natieve erwten/mais voer, maar dat de toevoeging van extra eiwit de voederconversie van kuikens op het intensief geperste erwten/mais voer van 1,76 naar 1,72 verlaagde. Dit betekent dat het positieve effect van een langzame zetmeelvertering (mede) door een betere eiwitbenutting wordt veroorzaakt.

In een derde groeiproef werden wederom een voer met snel verteerbaar zetmeel en een voer met langzaam verteerbaar zetmeel geformuleerd. Binnen deze voersamenstellingen werden vijf aminozuur (eiwit) niveaus ingebouwd. Dit leverde een proef op met 10 behandelingen (2 zetmeelvoeders x 5 aminozuurniveaus). Bij elk aminozuurniveau lieten de kuikens op de voeders met langzaam verteerbaar zetmeel een hogere groei en een lagere voederconversie zien. De verschillen waren echter het grootst bij de lage aminozuurniveaus. Deze resultaten bevestigen de hypothese dat een voer met langzaam verteerbaar zetmeel leidt tot een betere eiwitbenutting van vleeskuikens. De verschillen in voederconversie konden echter niet volledig aan een betere eiwitbenutting toegeschreven worden.

Fysiologische verklaring voor positief effect van LVZ op voerbenutting

Hoewel we op basis van onze proefresultaten geen sluitende verklaring voor het positieve effect van langzaam verteerbaar zetmeel kunnen geven, is het wel mogelijk om vanuit de fysiologie van het dier een aantal mogelijke effecten van langzaam verteerbaar zetmeel aan te dragen.
Zetmeel en eiwit worden tijdens de vertering afgebroken tot respectievelijk glucose en aminozuren. Glucose en aminozuren worden door de darmwand opgenomen via absorptie. Een deel van deze opgenomen stoffen wordt ter plekke in de darmwandcellen verbrand of omgezet in specifieke aminozuren. Ook wordt een deel van het opgenomen glucose omgezet in melkzuur. De rest wordt afgegeven aan het bloed. De verteringssnelheid van zetmeel bepaalt samen met de hoeveelheid door het dier opgenomen zetmeel de hoeveelheid glucose die per tijdseenheid wordt opgenomen in het bloed. Een verhoging van de glucoseconcentratie in het bloed resulteert in een insulineafgifte vanuit de alvleesklier naar het bloed. Onder invloed van dit insuline worden zowel glucose als aminozuren door lichaamscellen opgenomen, en zodoende kunnen de concentraties aan glucose en aminozuren in het bloed niet te hoog worden. Als na een maaltijd de absorptie van glucose veel sneller plaatsvindt dan de absorptie van aminozuren (bij een voer met snel verteerbaar zetmeel), dan is de insulinepiek al grotendeels voorbij als de aminozuren in de bloedbaan komen. Bij afwezigheid van insuline worden aminozuren niet door de cellen opgenomen en worden ze wellicht als overtollige voedingsstof verbrand. Dit heeft nadelige gevolgen voor de eiwitbenutting. Een snelle insulinepiek heeft ook een negatieve invloed op de benutting van glucose (en dus van energie). Glucose wordt gebruikt als energiebron voor veel lichaamsprocessen. Bij een snelle glucoseabsorptie is er al gauw een overmaat aan glucose en het overtollige glucose wordt dan vastgelegd in lichaamsreserves (glycogeen en vet). In situaties dat er dan een glucosetekort dreigt kan er weer glucose uit deze lichaamsreserves worden vrijgemaakt. Het is echter efficiënter om het glucose direct te benutten, dus niet via allerlei chemische omzettingen. Een voer met langzaam verteerbaar zetmeel zal tot een min of meer continue glucoseabsorptie leiden met als gevolg dat er meer glucose direct benut wordt. Een voer met langzaam verteerbaar zetmeel zal dus ook leiden tot een betere energiebenutting.
Zoals gezegd wordt een deel van de geabsorbeerde glucose en aminozuren in de darmwandcellen verbrand. Dit gebeurt om de darmwandcellen in hun energiebehoefte te voorzien. Bij een voer met snel verteerbaar zetmeel zal al het zetmeel in de eerste helft van de dunne darm verteerd zijn en door de snelle

absorptie van glucose zal de tweede helft van de dunne darm verstoken blijven van glucose uit het voer. Hierdoor zullen wellicht meer aminozuren uit het voer verbrand worden. Dit heeft een negatief effect op de eiwitefficiëntie. Als echter een voer met langzaam verteerbaar zetmeel gevoerd wordt, dan zal een deel van het zetmeel pas in de tweede helft van de dunne darm verteerd worden en hierdoor worden wellicht aminozuren gespaard die vervolgens gebruikt kunnen worden voor vleesaanzet en dus groei.

De boodschap

Op basis van de resultaten van dit project kunnen we dus concluderen dat een langzame zetmeelvertering in een betere voerbenutting resulteert dan een snelle zetmeelvertering. Verder hebben we gezien dat de snelheid van zetmeelvertering verschilt tussen grondstoffen en dat deze verhoogd kan worden door technologische behandelingen in de mengvoerfabriek. Dit betekent dat de pluimveenutritionist zich bewust moet zijn van het feit dat zetmeel van de ene grondstof niet zonder meer vervangen kan worden door zetmeel van een andere grondstof ('zetmeel ≠ zetmeel'). Zetmeel van de ene grondstof is slechter verteerbaar dan dat van de andere en de verschillen in verteringssnelheid zijn nog groter. Voor een langzame zetmeelvertering dient de pluimveenutritionist de juiste zetmeelbronnen te selecteren (bijvoorbeeld erwten en milo) en dient hij er zorg voor te dragen dat de positieve eigenschap van deze grondstoffen niet tenietgedaan wordt door intensieve hittebehandelingen in de mengvoerfabriek. De persoon die de perslijn bedient dient zich ook bewust te zijn van het feit dat hij de voederwaarde van het voer aanzienlijk kan beïnvloeden. Voor hem ligt er de uitdaging om een brok van een goede fysische kwaliteit te maken zonder dat de zetmeeltoegankelijkheid te veel verhoogd wordt. De ontwikkelde *in vitro* methode kan hierbij een handig controle-instrument zijn.

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Curriculum vitae

Roelof Eduard Weurding werd geboren op 6 september 1970 te Beilen. Na de lagere school (Prinses Beatrixschool te Beilen) ging hij in 1982 naar het Menso Alting College te Hoogeveen, waar hij in 1989 het Atheneum diploma behaalde. In zijn vrije tijd werkte hij op diverse agrarische bedrijven met pluimvee, droogbloemen en akkerbouw. Ook was hij regelmatig te vinden bij een dierenartspraktijk te Beilen. Zijn voornaamste hobby's zijn voetbal, fietsen en schaatsen op natuurijs. Na de middelbare school ging hij naar de Agrarische Hogeschool te Groningen (Van Hall Instituut). In de vier jaar van deze opleiding was hij werkzaam op een aantal melkveebedrijven in Nederland en Canada en liep hij stage bij een mengvoederbedrijf. Zijn afstudeerproject had betrekking op de veevoeding. Hij behaalde zijn diploma met lof in de afstudeerrichting Veehouderij in 1993. Vervolgens besloot hij aan de Landbouwuniversiteit van Wageningen veevoeding te gaan studeren en zich met name toe te leggen op mengvoedertechnologie. In het kader van zijn afstudeervak, dat hij aan de University of British Columbia in Canada uitvoerde, onderzocht hij de invloed van diverse technologische processen op de eiwitfermentatie van gerst en raapzaadschroot in de pens van melkkoeien. Een tweede afstudeervak deed hij aan de vakgroep Bedrijfskunde van de Landbouwuniversiteit. Na zijn afstuderen in 1996 was hij tot het einde van dat jaar als commercieel nutritionist werkzaam voor Verdugt B.V., een bedrijf dat organische zuren en zouten produceert. In januari 1997 werd hij aangesteld als promotie-assistent bij coöperatie ZON te Eindhoven. Hij werd gedetacheerd bij Stichting Instituut voor de Veevoeding 'De Schothorst' te Lelystad. Het onderzoek richtte zich op de zetmeelvertering van landbouwhuisdieren en de invloed van mengvoertechnologie hierop. Hij richtte zich met name op de zetmeelvertering van vleeskuikens en melkkoeien; het onderdeel vleeskuikens staat in dit proefschrift beschreven. Op 1 augustus 2001 trad hij als sectorspecialist rundvee in dienst bij coöperatie ACM te Meppel. De coöperaties ACM en Cavo Latuco zijn per 1 januari 2002 gefuseerd en de onderneming is sindsdien verder gegaan onder de naam Agrifirm.

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