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Philosophiae Doctor (PhD)
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Khaled Walid Itani

Norwegian University of Life Sciences
Faculty of Biosciences
Department of Animal and Aquacultural Sciences

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Supervisors

Birger Svihus
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
Postboks 5003 NMBU
N-1432 Ås, Norway

Margareth Øverland
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
Postboks 5003 NMBU
N-1432 Ås, Norway

Liv Torunn Mydland
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
Postboks 5003 NMBU
N-1432 Ås, Norway

Evaluation Committee

Øystein Ahlstrøm
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
Postboks 5003 NMBU
N-1432 Ås, Norway

Sebastian Kaczmarek
Department of Animal Nutrition
Poznań University of Life Sciences
Ul. Wołyńska 33
60-637 Poznań, Poland

Sanna Steenfeldt
Department of Animal Sciences
Aarhus University
Blichers Allé 20, Postboks 50
DK-8830 Tjele, Denmark

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Khaled Itani

Table of Contents

1. Abbreviation

The main abbreviations used throughout the present work are listed below. The rest of the abbreviations are described in the papers.

Cel-Ext Extruded diet containing fine cellulose

Cel-Pel Pelleted diet containing fine cellulose

FBS Faba bean starch

FCR Feed conversion ratio

HRSF High ratio of starch: fat

LRSF Low ratio of starch: fat

NDR Nitrogen disappearance rate

OH-Pel Pelleted diet containing coarse oat hulls

SNDR Starch to nitrogen disappearance rate

SBM Soybean meal

SDR Starch disappearance rate

W Wheat

WB Wheat-based

WS Wheat starch

2. Summary

Quantitatively, starch is the most important component and the major energy source in poultry diets. Although it is commonly believed that the broiler chicken has a large capacity to digest starch, numerous studies have demonstrated that starch digestibility in some cereal grains or legumes is in some cases suboptimal. In general, starch properties, processing method, inclusion level and the functionality of the gastro-intestinal tract have been considered factors affecting starch utilisation in poultry. To optimise all these factors, a better understanding of the mechanisms explaining the better starch utilisation in broilers is required. Thus, three experiments were conducted to study how gizzard function, starch inclusion level and different starch characteristics affect starch digestion dynamics in broilers.

In **experiment 1**, male broiler chickens were distributed to 48 cages (2 birds each) and given a wheat-based (**WB**) pelleted diet containing either coarse oat hulls or fine cellulose until d 19 to stimulate divergent development of the gizzard. Thereafter, both groups were further subdivided and challenged with a WB diet containing cellulose in either pelleted or extruded form on d 20 and 22. Either excreta or intestinal contents were collected at time intervals after feeding and analysed for digestibility marker and starch. Thus, in **paper I**, the hypothesis tested was that a rapid passage of digesta (caused by suboptimal gizzard function) will impair intestinal wheat starch (**WS**) digestibility when the starch has a low degree of gelatinisation (i.e. in pelleted diets). However, this problem can be alleviated by stimulating gizzard function or by increasing starch gelatinisation through extrusion. Results from **paper I** showed that starch degradation rate is associated with the flow of digesta, which is linked to gizzard development. In Addition, compared to gelatinised starch, enzymatic hydrolysis of intact starch granules may be limited with more rapid feed passage through the gut.

In **experiment 2**, mixed-sex broilers in 12 replicate pens were given isocaloric and isonitrogenous WB pelleted diets with either a high ratio of starch: fat (**HRSF**) or a low ratio of starch: fat (**LRSF**). The diets were formulated by replacing the isolated wheat-starch (**WS**) in the HRSF by an isocaloric mixture of rapeseed oil and sand in the LRSF. At d 17, the birds were challenged with *Eimeria* sp. in the drinking water to predispose them to intestinal proliferation of *Clostridium perfringens*. Ileal samples were collected on d 16 and 29. Thus, in **paper II**, the hypothesis tested was that a HRSF would impair starch digestibility compared to a LRSF particularly after intestinal infection. Contrary to our hypothesis, a lower ileal starch digestibility was observed in birds given the LRSF compared with the HRSF. The very

low fat content in the HSFR increased the friction in the pellet press, resulting in unintended higher pellet temperature and starch gelatinisation degree compared to the LSFR. For this reason, and due to the use of isolated WS (fine texture) in the HRSF, our data cannot be used to reject our hypothesis. Further work is required to clarify this research question, taking into consideration the potentially confounding roles of feed processing and physical form of starch sources.

In **experiment 3**, male broilers were distributed among four dietary treatments consisting of either wheat or faba bean as starch sources, and pelleting or extrusion as processing methods. The wheat (**W**) and the dehulled faba bean were finely ground using a pin mill. Subsequently, the faba bean was subjected to air classification to produce a faba bean starch-rich fraction (**FBS**). The WS or FBS was the sole starch source in the diets. Each dietary treatment was fed to 10 replicate pens. Thus, in **paper III**, the hypothesis tested was that in pelleted diets, legume starch will be more slowly digested compared to cereal starch. However, extrusion will reduce the difference in starch digestion rate and extent between the two sources. The hypothesis of the beneficial effects of a slow starch digestion or a low ratio of starch to nitrogen disappearance rate (**SNDR**) on broiler performance was also tested. Starch digestibility in the W was very high and similar regardless of the processing method. FBS was highly digestible, however, when used in pelleted diets, it had a lower digestibility and a slower disappearance rate compared to the W in all intestinal segments. The differences in digestibility between the WS and FBS were reduced with extrusion, resulting in an interaction between starch source and processing method. Neither feeding slowly digestible starch nor a lower ratio of SNDR improved feed conversion efficiency.

Overall, stimulating gizzard activity improves small intestinal functionality, i.e. starch digestibility, through a better digesta flow regulation. In other words, enzymatic hydrolysis of ungelatinised or large starch particles may be limited with more rapid feed passage to the small intestine. Fine grinding or a high degree of starch gelatinisation can overcome the resistant structural organisation of starch granules, increase enzyme accessibility and improve starch digestibility independent of the starch source, inclusion levels or gizzard function. Enzyme production and glucose absorption do not appear to be major limiting factors to the starch digestion process in broiler chickens. Accelerating starch digestion (or a high ratio of SNDR) had no detrimental effect on the growth performance of the birds, indicating that this hypothesis remains questionable.

3. Sammendrag

Kvantitativt er stivelse den viktigste komponenten og hovedkilden til energi i fjørfe-dietter. Selv om det vanligvis antas at broilerkyllingen har stor kapasitet til å fordøye stivelse, har mange studier vist at stivelsesfordøyeligheten av noen korn- og belgvekster i noen tilfeller er suboptimal, og at variasjonen mellom individuelle kyllinger kan være betydelig. Generelt har ulike stivelsesegenskaper, behandlingsmetode, inkluderingsnivå og funksjonalitet i tarmkanalen blitt vurdert som faktorer som påvirker stivelsesutnyttelsen hos fjørfe. For å oppnå en bedre stivelseutnyttelse hos broilere, kreves det en bedre forståelse av mekanismene bak alle disse faktorene. Det har derfor blitt utført tre kyllingforsøk for å studere kråsfunksjonen, stivelsesnivå i dietten og hvordan forskjellige stivelsestyper påvirker fordøyelsesdynamikken til stivelse i broilerkyllinger.

I **eksperiment 1** ble hanekyllinger fordelt på 48 bur (2 kyllinger per bur) og fôret med hvetebaserte pelleterte dietter som inneholdt enten grove havrekli eller fin cellulose fram til dag 19 for å gi ulik stimulering og dermed forskjellig utvikling av kråsen. Deretter ble begge gruppene ytterligere oppdelt og fôret med WB-dietter som inneholdt cellulose i enten pelletert eller ekstrudert form på dag 20 og 22. Tarminnhold og gjødsel ble samlet inn ved ulike tidsintervaller etter fôring og analysert for markør og stivelse. Hypotesen som ble testet i **artikkel I**, var at en raskere passasje av fôr gjennom tarmen (forårsaket av suboptimal kråsfunksjon) vil redusere fordøyelsen av stivelse i tarmen når stivelsen har en lav grad av gelatinisering (dvs. i pelleterte dietter). Dette problemet kan imidlertid reduseres ved å regulere passasjen av fôret gjennom stimulering av kråsen, eller ved å øke stivelsesgelatiniseringen ved ekstrudering. Resultater fra **artikkel I** viste at stivelsesnedbrytningshastigheten er assosiert med passasje av fôr gjennom tarmen, knyttet til utviklingen av kråsen. I tillegg sammenlignet med gelatinisert stivelse,at enzymatisk hydrolyse av intakte stivelsesgranuler kan begrenses med raskere fôrpassasje gjennom tarmkanalen.

I **eksperiment 2** ble kyllinger i 12 replikate binger gitt isokaloriske og isonitrogenholdige WB-pelleterte dietter med enten høy ratio av stivelse: fett (**HRSF**) eller lav ratio av stivelse: fett (**LRSF**). Diettene ble formulert ved å erstatte isolert hvetestivelse i HRSF med en blanding av rapsolje og litt sand for å gi samme energiinnhold i LRSF. Ved d 17 ble fuglene utfordret med *Eimeria* sp i drikkevannet for å predisponere dem for økt vekst av *Clostridium perfringens* i tarmen. Ilealprøver ble tatt på d 16 og 29. Hypotesen som ble testet i **artikkel II** var at HRSF ville redusere stivelsesfordøyeligheten, sammenlignet med LRSF, spesielt etter en tarminfeksjon. Men i motsetning til vår hypotese ble det observert en lavere ileal stivelsesfordøyelighet hos kyllinger fôret med LRSF, sammenlignet med HRSF. På grunn av det svært lave fettinnholdet i HSFR økte friksjonen i pelletspressen, noe som resulterte i utilsiktet høyere pelletstemperatur og grad av stivelsesgelatinisering sammenlignet med LSFR. Av denne grunn, og på grunn av bruk av isolert

stivelse (fin tekstur) i HRSF, kan ikke dataene våre brukes til å avvise hypotesen. Ytterligere arbeid er nødvendig for å klargjøre dette forskningsspørsmålet, tatt i betraktning de potensielt motstridende resultatene m.h.t. fôrprosessering og ulike fysiske former av stivelse.

I **eksperiment 3**, ble hanekyllinger fordelt på fire diettbehandlinger som bestod av enten hvete eller fababønner som stivelseskilder, og pelletering eller ekstrudering som behandlingsmetoder. Avskallede fababønner ble finmalt og luftklassifisert for å produsere en stivelsesrik fababønne-fraksjon (**FBS**), mens hveten (**WS**) ble finmalt før bruk. WS eller FBS var den eneste stivelseskilden i dietten. Hver diettbehandling ble fôret til 10 replikate binger. Hypotesen som ble testet i **artikkel III**, var at stivelse fra legumer vil bli saktere fordøyd, sammenlignet med stivelse fra korn, når gelatiniseringsgraden er lav (pelleterte dietter). Økning av gelatiniseringsgraden v.h.a. ekstrudering vil imidlertid redusere forskjellen i fordøyelseshastighet og omfang mellom de to stivelsekildene. Hypotesen om at saktere fordøyelse av stivelse, eller en lav ratio mellom stivelse- og nitrogen-fordøyelse (SNDR), ville gi fordelaktige effekter på veksten og fôrutnyttelsen hos kyllinger, ble også testet. Stivelsesfordøyeligheten av WS var veldig høy, og lignende for begge prosesseringsmetodene. FBS var svært fordøyelig, imidlertid når den ble brukt i pelleterte fôr hadde den lavere fordøyelighet og ble tatt opp langsommere opp fra alle segmentene i tynntarmen sammenlignet med WS. Forskjellene mellom WS og FBS ble redusert med ekstrudering, noe som resulterte i en interaksjon mellom stivelseskilde og prosesseringsmetode. Fôring med sakte fordøyelig stivelse forbedret ikke fôrkonverteringseffektiviteten, og heller ikke en lavere ratio av SNDR.

Generelt kan det konkluderes at ved økt stimulering av kråsaktiviteten forbedres tarmfunksjonen i tynntarmen, dvs. stivelsesfordøyeligheten, gjennom en bedre regulering av hastigheten til fôret som kommer til tarmen. Med andre ord kan enzymatisk hydrolyse av ugelatinisert eller store stivelsespartikler begrenses med for rask tilførsel av fôr som passerer gjennom tynntarmen. Fin malingsgrad eller høyere grad av stivelsesgelatinisering kan motvirkeden resistente strukturelle organisasjonen av stivelsesgranulater, øke enzymtilgjengeligheten og forbedre stivelsesfordøyeligheten, uavhengig av stivelseskilden, inkluderingsnivåer eller kråsfunksjon. Enzymproduksjon og glukoseabsorpsjon ser ikke ut til å være en stor begrensende faktorer for stivelsesfordøyelsesprosessen hos slaktekyllinger. Raskere stivelsefordøyelse (eller en høy ratio av SNDR) hadde ingen negativ effekt på vekstytelsen hos slaktekylling, noe som indikerer at denne hypotesen ikke kan bekreftes.

4. List of publications

The present thesis is based on three papers listed below. The papers will be referred to by their roman numbers throughout the thesis.

Paper I was published online on 9 January 2019 in the journal of British Poultry Science.

Paper II is based on a collaboration with The Veterinary Institute of Norway, in the project "Rearing broiler chickens without in-feed anticoccidials". This paper (part 1) will be submitted to the same journal together with a second related paper (part 2, Manuscript in preparation, Granstad *et al.* 2019).

Paper III is the first of two papers, currently in manuscript to be submitted for publishing in 2019.

Paper I: **K. Itani**, B. Svihus, 2019. Feed processing and structural components affects starch digestion dynamics in broiler chickens. British Poultry Science. DOI: https://doi.org/10.1080/00071668.2018.1556388.)

Paper II: **K. Itani**, S. Granstad, , L.T. Mydland, M. Kaldhusdal, B. Svihus. Varying ratio of starch to fat in broiler diet: 1. Effects on nutrient digestibility and production performance. (In manuscript)

Paper III: **K. Itani**, J.Ø. Hansen, B. Kierończyk, A. Benzertiha, A.E. Kurk, P.P. Kurk, F. Sundby, L.T. Mydland, M. Øverland and B. Svihus. Interaction between starch source and degree of starch gelatinisation in broiler chickens: Effects on starch degradation rate and growth performance. (In manuscript)

5. General introduction

Cereal grains are successfully used as the main energy source in commercial broiler diets. This energy is predominantly derived from starch and supplies more than half of the metabolisable energy required by the broiler chicken (Svihus 2011b). Consequently, optimal starch utilisation is critical, because any reduction or variability in starch digestibility will negatively affect the energy available to the bird (Mateos et al. 2002, Wiseman 2006) and impair feed efficiency. Commercially, pelleting is the dominant manufacturing process in the production of broiler feeds. During pelleting, only a limited amount of starch will gelatinise: thus, starch is to a large extent present as intact, hard-to-digest starch granules (Svihus 2014b).

Poultry species, including broiler chickens, have a shorter digestive tract relative to mammals (Denbow 2015) and a shorter retention time of digesta in the intestine (3 h) (Weurding 2002, Svihus 2014b, Liu et al. 2017) as compared to, for example pigs (4-10 h) (Van Leeuwen and Jansman 2007, Wilfart et al. 2007). In addition, the modern broiler has an impressive appetite, consuming on average 10% of its weight per day (Svihus 2014a) and can grow by 50-fold in almost five weeks (Choct 2009). Despite the high feed intake and short intestinal retention time of digesta, ileal digestibility of nutrients in general appears to be uncompromised in older broilers (Batal and Parsons 2002, Huang et al. 2005, Thomas et al. 2008, Tancharoenrat et al. 2013). With the exception of some physiological limitations in young broilers, the digestibility of starch, particularly ungelatinised, increases with age (Batal and Parsons 2002, Hetland et al. 2002, Svihus et al. 2004b, Zelenka and Ceresnakova 2005) and in some cases exceeds that of pigs (Huang et al. 1997, Willamil et al. 2012). This high ability to utilise starch is presumably due to sufficient amylase secretion (Moran 1982, Wiseman 2006, Svihus 2014b), high activity levels of disaccharidases shortly after hatching (Chotinsky et al. 2001) and a highly adaptive intestinal mechanism for glucose uptake (Suvarna et al. 2005).

Nevertheless, starch in wheat-based (**WB**) pelleted diets has been observed to be poorly digested by broiler chickens, with values ranging from 0.760 to 0.930 (Svihus 2001, Svihus et al. 2004a, Amerah et al. 2009, Svihus et al. 2010, Abdollahi et al. 2011). Poor starch digestibility has generally been attributed to the soluble fibre fraction present in wheat (**W**) (Annison 1993), W hardness (Carré et al. 2002) and resistant cell wall material (Meng et al. 2005). Fine grinding of hard W (Péron et al. 2005) or the addition of fibre-degrading enzymes to WB diets has only partially alleviated the problem of low starch digestibility (Svihus 2001). For instance, starch digestibility in enzyme-supplemented WB diets remained low compared to oat or barley-based diets without enzymes (Svihus 2001) and, in other cases, no relationship between grain hardness and starch digestibility was found (Rogel et al. 1987, Amerah et al. 2007). These inconsistencies suggested that other, possibly bird-related factors interfere with starch digestibility in pelleted W diets.

The gizzard is the pacemaker of normal gut motility (Duke 1994) and the major site for particle size reduction and peptic proteolysis (Shires et al. 1987). Accordingly, shorter retention time in this compartment implies less physical and chemical breakdown of digesta and inadequate starch degradation along the intestinal tract (Svihus 2011b). It is well established that gizzard activity and size are highly influenced by diet structure. Numerous researchers have shown that feeding pelleted diets reduced the grinding activity and thus the relative weight of the gizzard compared to diets containing coarse or large particles (Engberg et al. 2002, 2004, Amerah et al. 2009). Moreover, Svihus (2006) observed that feed intake was negatively correlated with energy utilisation, particularly in birds fed diets that did not stimulate gizzard activity. In addition, Svihus (2011b) reported that starch digestibility in pelleted WB diets was correlated with the relative empty gizzard weight, as all birds with less developed gizzards exhibited low starch digestibility. Corroborating this, wood shaving inclusion (60 g/kg) (Amerah et al. 2009) or the addition of oat hulls (100 g/kg) (Hetland et al. 2003) as gizzard-stimulating components in a WB diet, improved ileal starch digestibility (respectively 0.94 vs 0.85 and 0.99 vs 0.97) compared to the control diet. In a previous study, Svihus and Hetland (2001) indicated that an overload of wheat starch (**WS**) in the ileum, due to high feed intake, was the cause of reduced starch digestibility in birds given pelleted WB diet as compared to those fed a diet with whole W. Accordingly, it was proposed that a well-stimulated gizzard may have a regulatory effect on the flow of digesta through the digestive tract and therefore on starch availability. In the same study (Svihus and Hetland 2001), the negative effects of high feed intake and high WS in the ileum, were alleviated when the control diet was diluted with 100 g/kg cellulose powder. As a result, starch digestibility increased from 0.79 in the control diet to 0.93 in the cellulose-diluted diet.

Similar to pelleted WB diets, low starch digestibility values were also noted in diets containing unprocessed legumes, with values ranging from 0.607 to 0.909 in, for instance, faba bean- or peas-based diets (Carre et al. 1991, Lacassagne et al. 1991, Hejdysz et al.

2016a). Grain legumes such as faba bean (*Vicia faba*) are considered good source of nutrients and energy for poultry: however, their use in broiler diet has been limited to partial replacement due to their lower protein content compared to soybean meal (**SBM**), and to the presence of several anti-nutrients (Jezierny et al. 2010) associated with reduced nutrient digestibility/ poor broiler performance (Wareham et al. 1991, Flores et al. 1994, Helsper et al. 1996, Vilariño et al. 2009). In addition, as described by Carré (2004), the hardness of legume seeds (attributed to the tight cell wall organization of cotyledons) would result in a high proportion of coarse particles after grinding and thus an accessibility problem to starch in the intra-cellular spaces of the particles (Longstaff and McNab 1987). Moreover, the starch granule's crystal structure type (A, B and C) may influence starch digestion (Zhang et al. 2006, Ao et al. 2007). Thus, *in vitro* studies have shown that type C starch from legumes is more slowly, and to a lesser extent digested than type A starch from cereals (Weurding 2002, Hoover and Zhou 2003, Sun et al. 2006, Li et al. 2018). Corroborating the *in vitro* studies, *in vivo* experiments with broilers have shown that starch from legumes is generally more resistant to intestinal degradation than cereal starch (Yutste et al. 1991, Weurding et al. 2001). Such experiments also confirm the ratio of amylose to amylopectin is higher in fava bean compared to wheat (Bhatty 1974, Madhusudhan and Tharanathan 1995, Grant et al. 2002), and high amylose content is associated with reduced starch digestibility in broilers (Rooney and Pflugfelder 1986, Gutierrez-Alamo et al. 2008). Compared to the more branched amylopectin molecule, amylose has a more compact and linear structure and thus, a lower surface area available for amylolytic action (Thorne et al. 1983).

Based on the general assumption that enzymatic secretion and glucose absorption are not major limiting factors in broilers as mentioned earlier, it can be postulated that other factors may impede enzymatic access to starch granules. Thus, regardless of the starch source, and as opposed to the mild pelleting processing, a more intense feed treatment, for instance extrusion, may be required to rectify the problem of accessibility and increase starch susceptibility to amylase (Qi and Tester 2016). In fact, extrusion has been shown to significantly increase the digestibility of legume starch in broilers, mainly as a consequence of increased starch gelatinisation (Hejdysz et al. 2016a, Hejdysz et al. 2017). According to Lund (1989), starch gelatinisation is the irreversible collapse or disruption of the molecular order of the starch granules when heated in the presence of water. The effects of increased gelatinisation on WS digestibility in broilers were however inconsistent. Plavnik and Sklan (1995) for example, observed no difference in the digestibility of starch between extruded and untreated WB diets. On the other hand, Zimonja and Svihus (2009) found that, compared to cold or steam pelleting, extrusion processing significantly improved ileal starch digestibility, indicating that this process resulted in more severe destruction of the starch granules.

While high ileal starch digestibility is always desirable, it has been proposed that feeding gradually or slowly digestible starch may improve the efficiency of feed conversion in broilers (Weurding 2002, Del Alamo et al. 2009, Liu and Selle 2015, Truong et al. 2015). These researchers hypothesised that rapidly digested starch (digested by the end of the jejunum) or, a high ratio of starch to nitrogen disappearance rate (SNDR), would not provide enough energy in the form of glucose to the enterocytes in the lower part of the small intestine compared to slowly digested starch. Consequently, a larger proportion of amino acids will be used as an energy source for the enterocytes rather than for muscle growth. Gradually digested starch (digested at the distal ileum) may thus spare amino acid oxidation due to its longer supply of glucose, resulting in improved growth performance of the bird (Weurding et al. 2003b). To manipulate the rate of starch digestion, two sources of starch with different characteristics, e.g. cereal vs legumes (Weurding et al. 2001) can be used, or the same starch source can be subjected to either pelleting or extrusion. These alternative approaches alter starch properties differently and impact the starch digestion profile (Zimonja and Svihus 2009).

As can be seen from the above introduction, starch source and properties, feed processing method and gizzard function will have great influence on the digestion and utilisation of starch in broiler chickens. Thus, a more detailed understanding of these factors is required. In the following sections, starch composition and structure will first be presented. Secondly, a short overview will be given on the common feed processing methods, in addition to the changes in starch during feed processing, namely gelatinisation. Thirdly, the starch digestion process will be reviewed and finally, the technique used to assess intestinal starch digestibility in broilers will be described.

5.1. Starch composition

Starch is an abundant plant polysaccharide (glucan) and the main form of stored energy in cereal and legume seeds, fruits, tubers and roots. Native or unprocessed starch is produced in the form of semi crystalline granules (**Figure 1**), built up in concentric crystalline and amorphous layers (**Figure 2**). Starch granules vary in size (from less than 1 µm to more than 100 µm), shape (spherical, oval, polygonal, etc.) and chemical composition depending on the type of the species (Swinkels 1985, Raeker et al. 1998, Lindeboom et al. 2004, Jane 2006, Wani et al. 2016). Starch consists of two distinct, cold water-insoluble glucose polymers, amylose and amylopectin (**Figure 3**), which when combined represent approximately 98–99% of the dry weight (Tester et al. 2004). Although generally, amylopectin is the main constituent of the starch granule, representing around 70 to 80% of the total weight, the ratio of amylose to amylopectin can vary according to the starch source. For instance, 'waxy' starches contain as low as 1% amylose (Parker and Ring 2001, Jin et al. 2018), and starch from high-amylose starches may contain up to 70% amylose (Svihus et al. 2005). Amylose is essentially linear, containing 1% α-(1:6) and 99% α-(1:4) glycosidic bonds that form double and single helices in the native state of the granule (Tester et al. 2004). It has been suggested that a large portion of the amylose lie within the amorphous growth layers (Jenkins and Donald 1995). Amylopectin is much larger than amylose, and has a highly branched structure, containing around 5% α-(1:6) linkages in the branching points in addition to α-(1:4) bonds in the linear chains (Gallant et al. 1992). Amylopectin gives native starch its crystallinity (Veregin et al. 1986, Eliasson 2017) due to the clusters of double helices in the crystalline region. The double helices associate in pairs and are created by the intertwining of glucose chains within the amylopectin molecule (Oates 1997). Amylopectin branching points fall within the amorphous layers as indicated by Jenkins and Donald (1995) and Eliasson (2017). By X-ray diffraction studies, three forms of crystalline structure of amylopectin can be distinguished in the native starch granule (Biliaderis 1991, Sun et al. 2015). A-type pattern is a characteristic of cereal starches (rice, wheat, and corn). B-type pattern is found in tubers (potato), and C-type pattern which is present in legume seeds (pea and fava bean); reported by Gernat et al. (1990) and Cockburn et al. (2015) to be a mixture of A and B structures. According to Imberty et al. (1991), the differences between A and B structures arise from water content and the arrangement of the double helices in each structure.

The packing of the double helices in type-A is relatively compact with a low water content, while B-type has a more open structure with a hydrated helical core (Tester et al. 2004). In addition, starch granules contain other minor non-starch components such as lipids, protein and minerals, which as reviewed by Svihus (2005), may have an impact on starch behaviour during for example, feed processing or digestion.

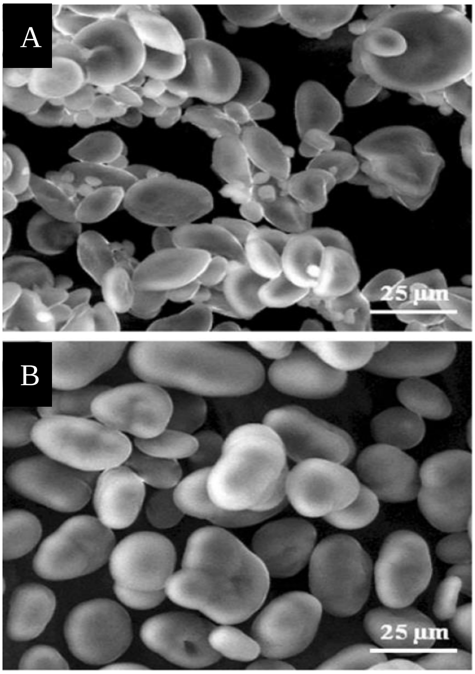


Figure 1. Environmental Scanning Electron Microscopy images of starch granules isolated from durum wheat semolina (**A**) and faba bean flour (**B**). Adapted from Petitot et al. (2010) and reprinted by permission from Springer Nature, copyright 2010.

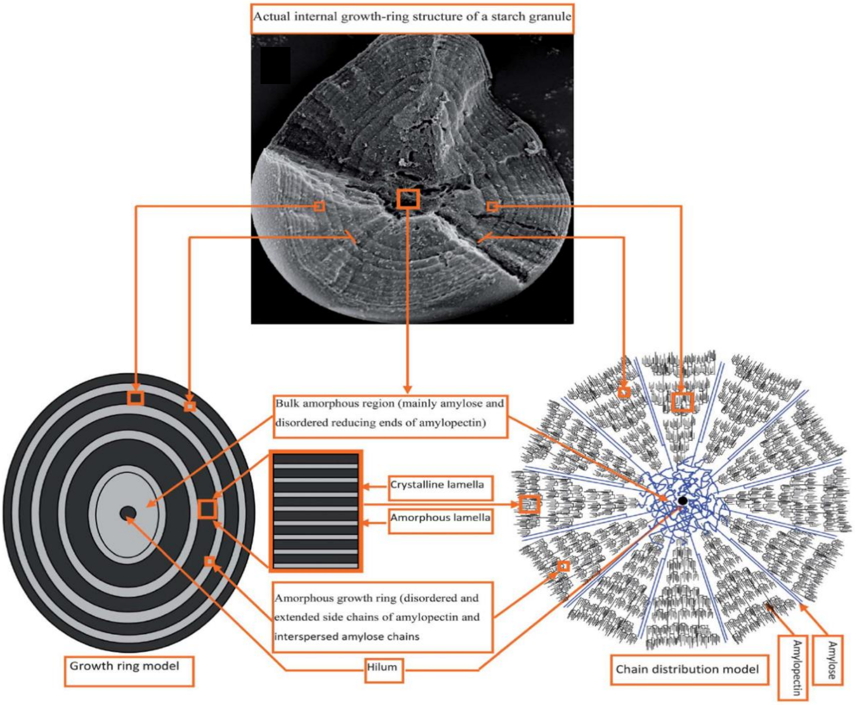


Figure 2. Model of starch granule organisation. Figure reproduced from Wang and Copeland (2013), modified from a figure in Wang et al. (2012). Reprinted with permission from Elsevier, copyright 2012.

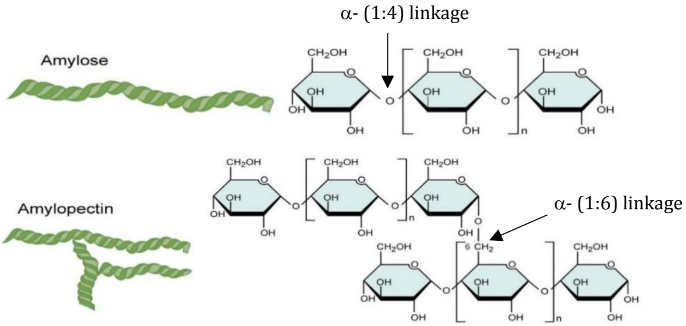


Figure 3. Starch amylose and amylopectin structure. Adapted from Sanyang et al. (2018) and reprinted by permission from Springer Nature, copyright 2018.

5.2. Feed processing and starch gelatinisation

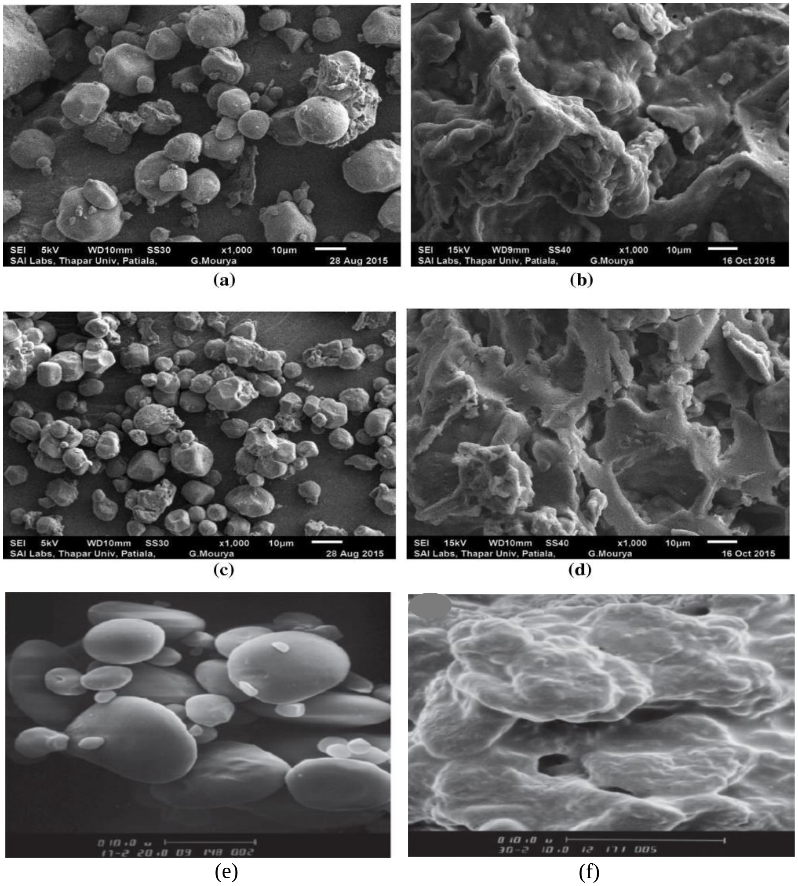
Feed processing refers to the treatment or alteration of the feed or feed ingredients prior to consumption by the animal (Maier and Bakker-Arkema 1992). The main objective of feed processing is to increase the nutritional value of the feed, for example through heat-induced changes in starch and protein, in order to improve and maximise the growth performance of the animal (Mateos et al. 2002). Feed treatment comprises thermal and cold (non-thermal) applications (Dehghan-Banadaky et al. 2007), which synergistically influence the physical, chemical, hygienic and nutritional characteristics of the feed (Sloan et al. 1971, Cox et al. 1986, Tillman and Waldroup 1987, Amerah et al. 2011, Svihus and Zimonja 2011). Cold processing has no external heat-addition, as in the case of particle size reduction (grinding) and blending of ingredients (mixing). Steam pelleting, extrusion and expansion, are the most common thermal (hydrothermal) processes used in feed production. Essentially, during these processes, small feed particles are agglomerated into larger particles by means of mechanical compression in combination with moisture, heat, shear force and pressurised steam (Abdollahi et al. 2013). The agglomerated mass of feed reduces selective feeding (Behnke 1996), thus ensuring the delivery of all micro- and macro-ingredients in one densified granule.

As described by Lund and Lorenz (1984), when starch is heated in excess water, starch granules begin to swell due to water uptake in the amorphous regions. The swelling is initially reversible but when a temperature threshold is reached, the swelling become irreversible and the stress on the crystalline regions increases. At a certain point in the swelling process, the crystalline regions are rapidly broken (Svihus et al. 2005) due to the disruption of intra- and inter-molecular hydrogen bonds and to the dissociation and unwinding of the double helices (Donald et al. 2001, Liu et al. 2002). Finally, starch granule loses its crystallinity and amylose leaches out. This process is termed gelatinisation. The destruction of the granular structure following gelatinisation (**Figure 4**) facilitates amylase access to the granule and increases starch susceptibility to hydrolysis (Svihus et al. 2005, Hejdysz et al. 2016a, Hejdysz et al. 2017). At excess water content, gelatinisation starts at 50 - 70 ◦ C (Svihus et al. 2005). However, when water or other solvent is limiting, gelatinisation temperature will be inversely related to water content (Donald et al. 2001), and thus more heat or mechanical energy will be needed to plasticise the amorphous regions and to promote gelatinisation (Rooney and Pflugfelder 1986, Svihus et al. 2005). For instance, Burros et al. (1987) found that in a limited water-system, pressure and physical shear were important contributors to starch degradation, as it allowed for faster water transfer into the interior of the starch molecule.

Several researchers reported that starch gelatinisation can be also modified, delayed or inhibited by the presence of lipids (Eliasson et al. 1981, Lund and Lorenz 1984). Due to its hydrophobic nature, fat may interfere with the hydration of feed components, for example by coating starch granule and limiting steam penetration (Zimonja et al. 2007). As reported by Putseys et al. (2010), the amylose helix is hydrophilic on the outside, but has a hydrophobic cavity, which favours the formation of hydrophobic interactions, particularly with the aliphatic hydrocarbon tail of the lipid (López et al. 2012). As a result of this complexing, fat may hinder the transport of amylose from the granule to the water, consequently repressing swelling and solubilisation (Eliasson et al. 1981, Svihus et al. 2005).

A low extent of starch gelatinisation in conventional pelleting (between 10 and 200 g starch/kg) is often reported (Svihus et al. 2005, Svihus and Zimonja 2011) due to the low total water content (14-16%) and moderate temperature (75-85 °C) during this processing. Contrary, the combination of higher moisture content (around 30%) and temperature (up to 120 °C) during extrusion may result in more severe and sometimes complete destruction of starch structure and thus greater extent of gelatinisation (Lund and Lorenz 1984, Hoover 1995, Zimonja and Svihus 2009, Boroojeni et al. 2016). In addition, the extrudate is exposed to high pressure in combination with more severe shear force. Consequently, and depending on the processing conditions employed, these processes may: 1) generally result in a different extent of physico-chemical alterations in the starch (gelatinisation), protein (denaturation) and other feed components, and 2) have beneficial effects on the nutritional value of the diet through increased availability of protein and starch, or detrimental effect through the destruction of heat-labile components like some amino acids, exogenous enzymes and vitamins and/or the formation of some enzyme-resistant Maillard products (Sørensen et al. 2002, Svihus and Zimonja 2011, Abdollahi et al. 2013).

Pelleting is the dominant manufacturing process in the production of broiler feeds (Cutlip et al. 2008), while extrusion on the other hand, was used to a lesser extent because of its high initial investment costs and inconclusive results (Jones et al. 1995, Plavnik and Sklan 1995, Moritz et al. 2005). Nevertheless, extrusion has recently received renewed interest for its beneficial effects on starch, protein and energy availability, and performance of broilers fed on diets containing less commonly used novel feed ingredients (Hejdysz et al. 2016a, Hejdysz et al. 2016b, Rutkowski et al. 2016, Hejdysz et al. 2017).



(a) (b)

(c) (d)

(e) (f)

Figure 4. Scanning electron microscopy images of (**a**) native millet starch granules, (**b**) gelatinised millet starch, (**c**) native sorghum starch granules, (**d**) gelatinised sorghum starch, (**e**) native wheat starch granules and (**f**) gelatinised wheat starch.
(**a**), (**b**), (**c**) and (**d**) are adapted from Alabi et al. (2018) and reprinted by permission from Springer Nature, copyright 2017. (**e**) and (**f**) are adapted from Zhou et al. (2014) and reprinted by permission from John Wiley and Sons, copyright 2014.

5.3. Intestinal starch digestion

During digestion, starch is first broken down by pancreatic amylase into smaller fragments, which are subsequently hydrolysed by enzymes (disaccharidases) located on the brush-border membrane of enterocytes to yield absorbable glucose, the basic unit of starch. Unlike other monogastric animals like pigs, chickens lack teeth for mastication, and do not secrete salivary amylase. Thus, chickens swallow their food immediately with no considerable physical or chemical changes. Depending on the feeding regimen, feed may bypass the storage compartment (crop) as indicated by Svihus (2014a) or may be stored temporarily to be hydrated before passing to the stomach. Although no digestive enzymes are secreted by the crop (Classen et al. 2016), longer retention of large quantities of feed in this storage pouch, for example through the use of intermittent feeding (Svihus et al. 2010), increases fermentation and lowers the pH. This creates favourable conditions for enzymes, both, exogenous (Zeller et al. 2016) and of microbial origin (Bayer et al. 1975). Yet, the crop seems to play a limited role in starch digestion given the small amount of feed retained there when the practice of *ad-libitum* feeding is predominant. In the stomach (proventriculus + gizzard), chemical and mechanical digestion initiates. Feed mixes with hydrochloric acid and pepsinogen, a precursor for the proteolytic enzyme pepsin that degrades proteins, thus, the protein matrix associated with, or shielding the starch granules. The gizzard receives, mixes and, due to its musculature, it crushes the acidified feed particles until reduced to an appropriate size. But, when feed particle-size does not stimulate gizzard activity, feed may be discharged more rapidly to the rest of the digestive tract (Sacranie et al. 2017). This may potentially result in an overload of inadequately digested material into the small intestine, and thus a reduction in nutrient digestibility (Svihus and Hetland 2001). The small intestine (duodenum, jejunum and ileum), is essentially the main site for digestion and absorption of nutrients. In the duodenum, bile and the alkaline pancreatic juices (containing α-amylase) are secreted to neutralise the acidic digesta from the gizzard to a pH of 6.5-7.5 (Svihus 2010), and to start the digestion process. Amylase, is an endo-enzyme that can only hydrolyse internal α-(1:4) glycosidic bonds in amylose and amylopectin, but has no specificity for the α-(1:6) linkages in amylopectin (Gray 1992). Also, amylase efficiency in cleaving α-(1:4) bonds decreases when approaching branching points (Carré 2004), particularly within the clusters due to steric hindrance (Park and Rollings 1994). Amylose is therefore broken down to one, two or more glucose residues, namely glucose, maltose and maltotriose (**Figure 5**). In addition to the latter, amylopectin's hydrolytic products also include various oligosaccharides containing a branch point, and are of at least four glucose units, called α–limit dextrins (Caspary 1992) (**Figure 5**). Maltose, maltotriose and the branched oligosaccharides are further hydrolysed into glucose by brush border enzymes, essentially maltase (α-glucosidase) and isomaltase (α-dextrinase). Maltase is an exo-enzyme that hydrolyses α- (1:4) bonds at the non-reducing end of maltose and maltotriose, while isomaltase cleaves the non-reducing end of α- (1:6) bond to produce maltose, maltotriose and glucose.

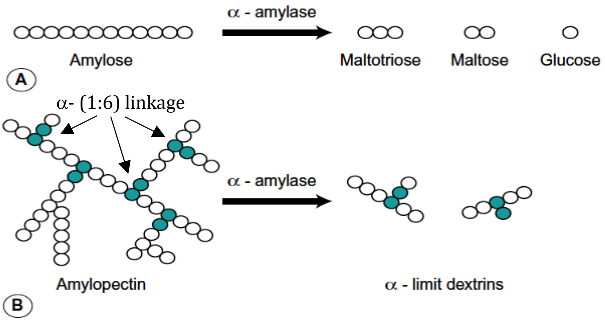


Figure 5. Degradation of (A) amylose and (B) amylopectin by α- amylase. Adapted from Smith and Morton (2010) and reprinted with permission from Elsevier, copyright 2010.

Glucose is mainly actively absorbed (Suvarna et al. 2005) and transported across the intestinal wall by an Na^+-dependent glycoprotein carrier (Braun and Sweazea 2008, Denbow 2015) located at the brush border of the enterocytes (**Figure 6**). Unlike in mammals, two molecules (instead of one) of Na^+, together with one molecule of glucose from the lumen bind to the carrier at the apical side and travel into the cell (Kimmich and Randles 1984). The driving force for this transport is the Na^+-K^+ pump located at the basolateral side which pumps the intracellular Na^+ ions against their electrochemical gradient to the basolateral side (out of the cell) while simultaneously pumping K^+ ions into the cell (Gray 1992) (**Figure 6**). Once inside the cell, glucose diffuses passively via a second carrier protein or by a Na^+ independent mechanism (Denbow 2015) from the basolateral side into the blood capillaries. Part of the glucose will be oxidized by the gut wall. The rest is taken via the portal vein and either stored as glycogen and fat or used as a readily available energy source for the tissues. Almost all of the glucose released from starch digestion is absorbed within the small intestine (Denbow 2015) with the majority (85%) being taken up in the duodenum and jejunum (Riesenfeld et al. 1980). The smaller fraction of glucose that is not absorbed in the upper intestine may be taken up by the ileum as reported by Levin et al. (1983).

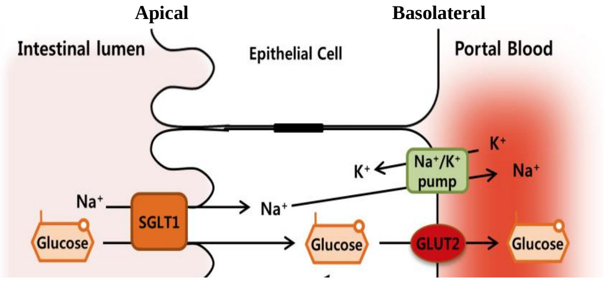


Figure 6. Model for glucose transport across the intestinal epithelium. Adapted from Lee and Cha (2018).

5.4. Assessing small intestinal starch digestibility in broilers

In vivo digestibility trials include the total collection method, which requires an accurate measurement of feed intake and a quantitative collection of excreta over a period of time (Choct 2016) or the slaughter method (the main method used in the experiments reported herein) in which the birds are killed after a period of access to feed, and representative digesta samples from the small intestine are collected and analysed (Knudsen et al. 2006). The slaughter method precludes the need to quantitatively measure feed intake and excreta output (Short et al. 1996) since an indigestible marker (Titanium dioxide, chromic oxide etc.) with known concentration is added to the feed. Nutrient digestibility can thus be calculated by the change in nutrient concentration between the diet and digesta sample, relative to the marker concentration in the diet and digesta sample. The slaughter method offers the possibility to collect digesta from any part of the small intestine and to obtain a general overview of the digestion dynamics of the starch along the intestinal tract (Weurding 2002). As described by Choct (2016), an ideal marker (indicator) is able to uniformly mix with the feed prior to ingestion and to follow the passage of the digesta along the digestive tract. The marker must be inert with no adverse effects of any sort on the animal. The marker must not be digestible or fermentable by the animal or the resident microflora. Finally, it should be used in small quantities and importantly, easy to analyse.

Although it is commonly believed that the broiler chicken has a large capacity to digest starch, numerous studies have demonstrated that starch digestibility in some cereal grains (like wheat) or legumes, is in some cases suboptimal, and that the variation between individual birds can be substantial. In general, starch properties, processing method, inclusion level and the functionality of the gastro-intestinal tract have been considered factors affecting starch utilisation in poultry. Therefore, an understanding of the mechanisms enabling better starch utilisation in broilers (when these factors are optimised) is required. The objectives of this study were based on this premise.

6. Objectives

The main aim of this thesis was to study how starch properties, starch inclusion level and different feed flow patterns affect starch digestion in broilers.

In order to achieve this, three experiments were conducted to test the following hypotheses:

1- a) A rapid passage of digesta will impair starch digestibility if the starch has a low degree of gelatinisation.

   b) The problem of low intestinal starch digestibility can be alleviated by regulating feed passage through gizzard stimulation, or by increasing starch gelatinisation through extrusion.

2- A lower dietary inclusion level of starch will reduce starch load in the gut, and improve starch digestibility compared to higher inclusion.

3- a) In pelleted diets, legume starch will have a slower digestion rate compared to cereal starch. However, extrusion will change the digestion profile of legume starch, making it as available as that of cereals.

   b) Pelleting will result in a low ratio of starch: nitrogen disappearance rate (**SNDR**) compared to extrusion, and may thus be beneficial for broiler performance as opposed to extrusion.

7. Summary of papers

7.1. Paper 1

Feed processing and structural components affect starch digestion dynamics in broiler chickens

A 2 × 2 factorial design was used to test the hypothesis that impaired intestinal starch digestibility is attributable to rapid passage of digesta from the gizzard to the intestine, and that, compared to steam pelleting, increasing the availability of starch through extrusion cooking may alleviate the potential negative effect of rapid digesta flow on starch utilisation. Thus, 7-d-old-broiler chickens were distributed to 48 cages and given a wheat-based (**WB**) pelleted diet containing either coarse oat hulls (**OH-Pel**) or fine cellulose (**Cel-Pel**) until d 19 to stimulate divergent development of the gizzard. Thereafter, both groups were further subdivided and challenged with a WB diet containing cellulose in either pelleted (Cel-Pel) or extruded (**Cel-Ext**) form on d 20 and 22. Either excreta or intestinal contents were collected at time intervals after feeding and analysed for marker and starch. OH-Pel increased gizzard size and holding capacity. No excessively high starch levels (maximum 25 g/kg) were detected in the excreta. However, 8 h feed-deprived birds given Cel-Pel and challenged with Cel-Pel exhibited higher starch excretion and showed large individual variation during the first 135 min of collection. Contrary to the OH-Pel group, more digesta and starch passed to the jejunum at 1 and 2 h and ileum at 2 and 3 h after feeding for birds given Cel-Pel, resulting in lower jejunal and ileal starch digestibility. Increased starch gelatinisation through extrusion processing significantly improved starch digestibility regardless of gizzard function. However, at 1, 2 and 3 h after feeding, more digesta was retained in the foregut of birds given Cel-Ext. The current data showed that starch degradation rate is associated with the flow of digesta which is linked to gizzard development, and that enzymatic hydrolysis of intact starch granules may be limited with more rapid feed passage through the gut.

7.2. Paper 2

Varying ratio of starch to fat in broiler diet: 1. Effects on nutrient digestibility and production performance

The hypothesis of this experiment was that a diet with a high ratio of starch to fat (**HRSF**) may impair nutrient digestibility and growth performance as compared to a diet with a low ratio of starch to fat (**LRSF**). From d 10 to 29, broilers in 12 replicate pens were given isocaloric and isonitrogenous diets with either a HRSF or LRSF, by replacing the isolated wheat-starch (**WS**) in one diet by a mixture of rapeseed oil and sand in the other diet. At d 17, a 10-fold dose of live vaccine strains of Eimeria species was administered in the drinking water to predispose the birds to intestinal proliferation of Clostridium perfringens. Ileal samples were collected on d 16 and 29. Weight gain did not differ among the treatments, however birds fed LRSF were less efficient in feed conversion as compared to those fed HRSF. Ileal starch digestibility tended to be higher at d 16 and was higher at d 29 for the HRSF group, while ileal energy digestibility was not affected by the treatments. The HRSF did not induce an overload of starch in the ileum. Accordingly, ileal starch digestibility was improved with increasing dietary starch level from 23 to 45 %, demonstrating the high capacity of the broiler chicken to digest high levels of starch even under challenging conditions. The inadvertently higher extent of starch gelatinisation and the use of isolated WS in the HRSF, as well as possible lipid-amylose interactions in the LRSF may have caused the increased starch digestibility in the HRSF. Therefore, our data cannot be used to reject the hypothesis that HRSF may impair digestibility and production performance. Further work is required to clarify this research question, taking into consideration the potentially confounding roles of feed processing and physical form of starch sources.

7.3. Paper 3

Interaction between starch source and degree of starch gelatinisation in broiler chickens: Effects on starch degradation rate and growth performance.

A 2x2 factorial design was used to test the hypothesis that in pelleted diets, legume starch will be more digestion-resistant as compared to cereal starch, and that, increased gelatinisation through extrusion would reduce the difference in starch digestibility and growth performance between the two sources. Additionally, the study allowed for testing the hypothesis of the beneficial effect of a more gradual starch digestion or a low ratio of starch to nitrogen disappearance rate (**SNDR**) on broiler performance. From 17 to 29 d, birds were randomly distributed among four dietary treatments consisting of either wheat (**W**) or faba bean starch fraction (**FBS**) as starch sources, and pelleting or extrusion as processing methods. Each treatment had 10 replicate pens with five birds per pen. Extrusion cooking resulted in a more extensive starch gelatinisation compared to the pelleting process, as expected. Birds fed W tended ($P < 0.082$) to have better feed conversion ratio (**FCR**) than those fed FBS, while the difference between processing methods was insignificant. As a result, there was no interaction between starch source and processing method on FCR. FBS in pelleted diet had lower starch digestibility and a slower starch disappearance rate compared to W in all intestinal segments ($P < 0.05$). The interaction between starch source and processing method in all intestinal segments ($P < 0.001$) demonstrated that FBS responded more to gelatinisation through extrusion than did the W. As a result, differences in starch digestibility between the W and FBS were reduced with extrusion. Feeding slowly digestible starch did not improve feed conversion efficiency, nor did a lower ratio of SNDR.

8. Main results and discussion

The main aim of this thesis was to study how gizzard function, starch inclusion level and different starch characteristics affect starch digestion dynamics in broilers. The three aforementioned hypotheses are discussed concurrently to link the three experiments, as opposed to separate discussions of each hypothesis. Taken together, the three experiments showed that the physico-chemical characteristics of the starch sources exerted a larger impact on starch utilisation and digestibility than gizzard function or starch inclusion level. Accordingly, the negative effects of: 1) poor gizzard functionality, 2) high starch inclusion level, or 3) digestion-resistant properties of legumes, were mitigated when the starch source was highly gelatinised or ground to a fine texture. In addition, given the observed high starch digestibility values particularly in extruded diets, it can be suggested that the enzymatic secretion and glucose absorption are less likely to be major limiting factors to starch digestion in broilers. Accelerating starch digestion had no detrimental effect on the growth performance or the efficiency of feed conversion of the birds, indicating that this hypothesis remains questionable, and factors like protein digestion rate and site must also be taken into consideration.

The prominent role of an optimally functioning gizzard in improving nutrient digestibility in general and starch digestibility in particular, is well-documented (Svihus 2011a). The improvement in starch digestion has been linked to a better grinding activity (more finely ground particles) and an extended retention time of digesta in the acidic environment (chemical degradation) of the gizzard (Hetland et al. 2002, Amerah et al. 2008, Mateos et al. 2012). Accordingly, it has been hypothesised that an active gizzard can regulate the flow of digesta into the small intestine in a way that does not compromise intestinal digestive and absorptive capacities (Svihus and Hetland 2001). However, when diets do not stimulate gizzard development and function, more inadequately broken-down feed components, for example ungelatinised starch in pelleted diets, may pass rapidly into the small intestine and potentially escape digestion (Svihus et al. 2010, Sacranie et al. 2017). The finding from **paper I** supports our first hypothesis, emphasising the importance of the gizzard as a feed flow regulator. The lack of gizzard stimulation was associated with a lower dry matter content in the gizzard, a more rapid digesta flow into the small intestine, and a lower intestinal starch digestibility.

The first hypothesis was not tested in **paper II**, as no dietary structural components were used. The two pelleted diets in **paper II** contained different starch levels, under the hypothesis that, a high (**HRSF**) inclusion level of starch (450 g/kg) is more challenging to digest compared to a low (**LRSF**) inclusion level (230 g/kg) in birds with intestinal infection. Since no coarse components were included as mentioned above, the negative effect of poor gizzard development on starch digestibility was expected to be larger with higher starch load in the gut (HRSF), particularly after *Eimeria* infection. However, and contrary to our second hypothesis, a lower ileal starch digestibility was observed in birds given the LRSF compared with the HRSF (on average 0.922 vs 0.964). Thus, despite the higher starch load in the gut, starch in the HRSF was more accessible to amylase. This is not in line with Svihus and Hetland (2001) who hypothesised that high concentrations of WS in the intestinal chyme is inversely related to starch digestibility. These researchers detected an increase in ileal starch digestibility (from 0.73 to 0.93) when a portion of the W (starch source) in a WB control diet was replaced by fine cellulose powder (100 g/kg). This discrepancy indicates that the differences in starch digestibility observed in **paper II** were attributed to starch properties rather than its concentration in the diet. For instance, being finely ground (isolated starch), having a higher extent of gelatinisation or being affected by other components such as lipids, as will be discussed later on.

In **paper III**, no coarse components were used in the diets, however, regardless of the starch source (cereal or legume) or the activity of the gizzard, ileal starch digestibility coefficients in pelleted diets were high and above 0.970. In **paper I,** the wheat was ground to pass through a 2mm screen, while in **paper II**, a 3mm screen was used and the isolated starch (HSFR) had a very fine texture. In **paper III**, the wheat and the faba bean were pin-milled. This method of grinding generally produces finer particle size distribution (Wu et al. 1990) compared to a hammer mill fitted with a 3mm screen (Svihus et al. 2004b) or even a 2mm screen (Péron et al. 2005). The preliminary conclusion from the above results is that when gizzard activity is suboptimal and pelleted diets are fed, the negative effect on starch digestibility may be alleviated if the starch source is isolated or very finely ground. This conclusion is in agreement with Gunawardena et al. (2010a, 2010b) who reported almost complete starch digestion, measured at the ileal and total tract levels in pigs fed isolated wheat or corn starch or air-classified faba bean starch concentrate. Similarly, Péron et al. (2005) found that fine grinding of hard wheat (6 vs 2mm hammer mill screen) significantly increased starch digestibility from 0.85 to 0.93 in broilers fed on a WB pelleted diet. To our knowledge however, there is lack of studies examining the effects of finer grinding (using pin mill) on WS digestibility in broiler chickens. Care must therefore be taken before such conclusions can be drawn because other factors may either confound or act in synergy on the digestion process of starch, as will be discussed further below.

The destruction of the starch granular structure following gelatinisation facilitates amylase accessibility and increases starch susceptibility to hydrolysis (Svihus et al. 2005, Hejdysz et al. 2016a, Hejdysz et al. 2017). This agrees with our first and third hypotheses. Although generally, ileal starch digestibility in pelleted diets was high and on average 0.944, increasing the degree of starch gelatinisation through extrusion offered room for improvement. Thus, independent of the starch source (cereal or legume), ileal starch digestibility in extruded diets (**paper I** and **III**) was not influenced by the flow dynamics of digesta, i.e. gizzard function, and was almost 5% higher than that of pelleted diets, averaging 0.989. While not intended, the two pelleted diets in **paper II** had different levels of starch gelatinisation (HRSF: 57% vs LRSF: 15%). The high percentage of gelatinised starch in the HRSF was unexpected, since pelleting usually results in a lower degree of starch gelatinisation as seen in **paper I** and **III,** and by others (Moritz et al. 2005, Zimonja and Svihus 2009). The average gelatinisation values in **paper I** and **III** were 245 and 878 g/kg of total starch for pelleting and extrusion, respectively. Due to its very low-fat content (14 g/kg), the HSFR diet resulted in greater mechanical shear in the pellet press, which increased frictional heat, temperature and consequently starch gelatinisation. Also, the use of isolated WS may have contributed to the unusually high gelatinisation, since the starch purification process eliminates almost all non-starchy components that can hinder water uptake and granule swelling (Dhital et al. 2017). Contrary, because of the higher fat content (95 g/kg) in the LRSF, and due to the lubricating properties of oil, there was a reduction in frictional heat and temperature in the die, and as a result, a smaller fraction of starch was gelatinised. As mentioned earlier, starch digestibility was significantly improved with extrusion as compared to pelleting due to a higher extent of gelatinisation (**paper I** and **III**). In **paper II**, starch digestibility was significantly higher in the HRSF compared to the LRSF, despite the higher starch content in the former diet. The results indicate that changing the availability of starch through feed processing will be beneficial as long as the semi-crystalline structure is disturbed or damaged enough to increase starch susceptibility to digestive enzymes. In other words, increasing the degree of starch gelatinisation will rectify the problem of reduced starch digestibility, demonstrating that, when present in a readily digestible form, starch digestion becomes less limiting even at high inclusion levels or under poor gizzard development.

The very high and almost complete starch digestion, particularly in **paper I** and **III,** indicates that amylase production/secretion and brush border disaccharidases activity may not be major limiting factors in broiler chickens (Moran 1982, Wiseman 2006, Svihus 2011b, Svihus 2014b). This is in line with Mahagna et al. (1995), Svihus and Hetland (2001), Kaczmarek et al. (2014) and Stefanello et al. (2015) who did not detect any change in starch digestibility following the addition of exogenous α-amylase to a corn-SBM or wheat-SBM based diet. Nevertheless, Gracia et al. (2003) and Amerah et al. (2016) found that amylase supplementation significantly improved ileal starch digestibility (on average by 1.3%) in broiler chicks fed on corn-SBM based diets, suggesting inadequate secretion of pancreatic amylase in some cases. The observed low starch digestibility in **paper II** in birds given the LRSF compared to the HRSF diet is at least partly in agreement with the latter suggestion. As there was a trend in the HRSF group to have higher amylase activity, the concomitant significant increase in starch digestibility in this group was not surprising. However, because of the unintended confounding effect of different level of starch gelatinisation as mentioned earlier, it is hard to speculate whether higher amylase concentrations were needed when the starch is minimally gelatinised. In fact, starch digestibility increased with age in the LRSF with no change in amylase activity. This suggests that, it is not the inadequate secretion of amylase per *se*, but it is the limited accessibility of amylase to the starch at younger age that may have caused the reduction in the digestibility. Because of the higher fat content in the LRSF, and due to the fact that lipids can form inclusion compounds with amylose in the intestine, a reduction in starch digestion *in vivo* (Holm et al. 1983) or *in vitro* (Cui and Oates 1999) may therefore be expected. On the other hand, if the proportion of fat remaining in the intestine is lower (higher fat digestibility at older age), less fat will complex with starch (Crowe et al. 2000), which improves the availability of starch for amylase hydrolysis. The concomitant improvement in starch digestibility with age in the LRSF group (0.894 at 16 d vs 0.950 at 29 d) is in line with this speculation, especially since amylase activity was similar at both ages. Generally, amylase activity was characterised by an increase or decrease depending on the amount of substrate in the digesta (**paper II** and **III**), and as demonstrated before (Karasov and Hume 1997). This physiological adaptation (Murugesan et al. 2014) may thus, at least partly, explain the high capacity of the birds to digest high levels of starch in the diet.

In addition, Sell et al. (1989) and more recently Kohl et al. (2017), concluded that poultry are able to modulate their intestinal enzymes according to diet composition, as they detected significantly higher disaccharidases activity in birds given starch-rich compared to low-starch diets.

Glucose absorption is also less likely to be limiting based on the very high ileal starch digestibility coefficients, particularly in birds fed extruded diets (**paper I** and **III**). The findings of Gilbert et al. (2007) and Suvarna et al. (2005) also corroborate this conclusion. The latter researchers observed that the intestinal capacity to absorb glucose increases with both, age and greater carbohydrate content in the diet, mainly as a result of an upregulation of glucose transporters in the small intestine. Also, although ileal digesta samples (**paper III**) were not washed with aqueous ethanol (80%) (to remove free sugars i.e. glucose) before starch analysis, starch concentrations in freeze-dried distal ileal digesta were still very low and not exceeding 28 g/kg following treatment with thermostable alpha-amylase and amylo-glucosidase (McCleary et al. 1994). Supporting this, Svihus (2011b) reported that the content of free glucose in ileal samples from birds exhibiting low WS digestion was never higher than 20 g/kg, while the undigested starch fraction could account to up to 300 g/kg.

It appears from the above that enzyme secretion and glucose absorption are not a major limitation for starch digestibility in pelleted diets. However, because of the clear advantage of extrusion in improving starch digestion, it is reasonable at least in part, to attribute the reduction in starch digestibility in pelleted diet to a more limited enzymatic accessibility to starch granules. This is supported by *in vitro* studies where, even when amylase concentration is adequate or present in high activity in digestive fluid, starch hydrolysis rate may still be limited by factors related to the physico-chemical properties of the starchy ingredient (Slaughter et al. 2001, Tahir et al. 2010) .

Weurding (2002), Del Alamo et al. (2009) and Liu and Selle (2015) hypothesised that feeding gradually or slowly digestible starch may improve the efficiency of feed conversion in broilers. These researchers proposed that rapidly digested starch would not provide enough energy in the form of glucose to the enterocytes in the lower part of the small intestine compared to slowly digested starch. Accordingly, a larger proportion of amino acids will be used as an alternative energy source for the enterocytes instead of for muscle growth (Weurding et al. 2003b). Results from **paper III** are not in accordance with this hypothesis, and this will be discussed further below.

According to Cant et al. (1996), the gastrointestinal tract uses around one fifth of the dietary energy for digestive and absorptive processes, and the largest portion of this energy is derived from amino acid catabolism rather than glucose (Fuller and Reeds 1998). This agrees with other reports where, glutamine and glutamate were reported to be more important oxidative substrates than glucose for the small intestinal mucosa (Souba 1993, Stoll et al. 1999, Reeds et al. 2000, Blasco et al. 2005). Given the above evidence, a significant portion of the dietary amino acids will thus inevitably be catabolised by the intestinal epithelium, and this will have important role on their availability to extra-intestinal tissues. Wu (1998) in particular, emphasised that the extensive catabolism of dietary essential amino acids in the first pass by the small intestine will significantly impair the efficiency of feed utilisation and performance of the animal. Interestingly, Van Der Schoor et al. (2001) found that in pigs fed low protein diet, the splanchnic tissues maintain a high rate of energy expenditure by increasing the oxidation rate of dietary glucose, thereby lowering the contribution of amino acids as metabolic fuel. Because broiler diets are usually adequate in protein and balanced for amino acids, the above may not be likely to occur if, for example, glucose availability, i.e. starch digestion rate is reduced, particularly in the jejunum, the major digestive and absorptive tissue (Gao et al. 2017). Li et al. (2008) investigated the effects of different starch sources on the appearance of amino acids and glucose in the portal circulation of pigs. They found that slowly digestible starch (resistant starch) significantly reduced glucose and amino acids net absorption into the portal vein. Accordingly, it was suggested that resistant starch may increase the catabolism of amino acids by the small intestine, which reduces the efficiency of nutrient utilisation and impair pig performance. In accordance with the findings of Li et al. (2008), feeding legume starch, a source of slowly digestible starch, tended to impair FCR compared to wheat starch, which was more rapidly digested (**paper III**). This is not in line with previous and recent suggestions (Weurding 2002, Liu and Selle 2015, Truong et al. 2015) about the beneficial effects of slowly digested starch on feed efficiency of broilers. It should also be mentioned that, although pelleting resulted in a numerically lower FCR compared to extrusion, the difference was not statistically significant ($P = 0.2093$). According to the hypothesis of negative effect of rapidly digested starch on feed efficiency (Weurding et al. 2003a), extrusion should have resulted in significantly poorer FCR compared to pelleting, but as stated above, this was not the case. Corroborating this, Karunaratne et al. (2018) found that rapid starch digestion resulted in a better feed efficiency based on the observed positive correlation between gain: feed ratio and starch digestibility in the upper and lower jejunum for broilers fed WB diets in mash form. Weurding et al. (2003a) on the other hand, reported that feeding pea-corn based diets (slowly digestible starch of a digestion rate of 1.05 h^{-1}) for broilers resulted in 1.9% improvement in FCR compared to feeding tapioca-corn diets (rapidly digestible starch of a digestion rate of 1.99 h^{-1}). Contrary, Del Alamo et al. (2009) found that feeding diets with rapid starch digestion rates (k_d) of 2.17 h^{-1} and 2.56 h^{-1}, resulted in improved growth rate and lower FCR values (1.572 and 1.579 respectively), as compared to young broilers fed a diet with lower starch digestion rate (k_d = 1.8 h^{-1} and FCR= 1.668). Also, Hejdysz et al. (2017) found that feeding pea in extruded form (up to 500 g/kg diet) improved broiler performance, nutrient and energy utilisation and FCR compared to raw form. Moreover, although apparent metabolisable energy (AME) was not measured in **paper III**, Truong et al. (2016) reported that slowly digestible starch may improve AME and nitrogen corrected AME (AMEn). However, more recent experiment from the same lab showed significant improvement in AME, ME:GE ratio, N retention and AMEn with 45% inclusion of rapidly digested purified maize-starch in a maize-SBM based control diet (Moss et al. 2018).

In several studies conducted at the same institution, different conclusions were derived regarding the relation of starch: nitrogen disappearance rate (**SNDR**) and broiler performance. As stated by Sydenham et al. (2017), some studies found that broiler performance improved linearly with a lower ratio of SNDR, while the same article (Sydenham et al. 2017) concluded that this relationship is quadratic, and emphasised the importance of an optimal balance between the digestive dynamics of the two components in the proximal jejunum. Conversely, Truong et al. (2017) did not detect any significant difference in any of the performance parameters between broilers fed six varieties of sorghum exhibiting different ratios of SNDR in all intestinal segments. In **paper III**, pelleting had a lower ratio of SNDR compared to extrusion, particularly in the proximal and distal jejunum, however, no significant difference in FCR was detected. Gilbert et al. (2007) concluded based on the expression levels of nutrient transporters that, the jejunum is the primary site of sugar assimilation in the chicken intestine, while the ileum is a more important site for amino acid assimilation. Thus, a more rapid starch digestion (higher ratio of SNDR) would be logical to meet the higher energy demands of the jejunum. This may simultaneously spare more amino acids from oxidation, thus increasing their appearance in the portal circulation, as seen recently by Yin et al. (2019). Consequently, a smaller portion of the amino acids will be used as fuel for the enterocytes in the ileum, a relatively less demanding tissue in terms of digestion and absorption compared to the jejunum (Gao et al. 2017). Clearly, the findings are inconsistent and contradictory due to the complexity of the hypothesis and to the presence of confounding factors. Further well-designed experiments are needed to clarify and to understand the relationship between the digestive dynamics of starch/nitrogen and its effect on broiler performance.

9. Concluding remarks, limitations and future perspectives.

It can be concluded from the experiments carried out in the present thesis that:

1. Stimulating gizzard activity improves small intestinal functionality i.e. starch digestibility, through a better regulation of digesta flow. In other words, enzymatic hydrolysis of intact (ungelatinised) or large starch particles may be limited with more rapid feed passage through the gut.

2. Fine grinding or a high degree of starch gelatinisation (for example through extrusion) can overcome the resistant structural organisation of starch, and improve both enzyme accessibility to starch granules and starch digestibility independent of the starch source, inclusion levels or gizzard function.

3. Enzymatic production and glucose absorption do not appear to be major limiting factors to the starch digestion processes in broiler chickens.

4. Accelerating starch digestion had no detrimental effect on the growth performance or the efficiency of feed conversion of the birds, indicating that this hypothesis remains questionable, and factors like protein digestion rate and site must be also taken into consideration.

In each of the three experiments reported herein, attempts were made to eliminate or reduce potential confounding factors, particularly when formulating the experimental diets. For this reason, and with the exception of **paper II**, two identical wheat-based diets were formulated to contain either a coarse or fine fibre source (**paper I**) and two diets were formulated to contain either wheat or bean as the sole starch sources (**paper III**). Due to the inherent compositional difference of the starchy feedstuffs (wheat and bean), the diets were balanced for amino acids. This way, any observed effect can be attributed to the single variable in the diets. However, due to the different feed processing methods and diet composition (**paper II**), unintended minor differences between and within processed diets (extruded and/or pelleted) were unavoidable (physical characteristics of the pellets). Still, birds in all experiments had normal to high feed intake, indicating that the minor physical differences in the pellets had no effects on this parameter. Also, different processing conditions will alter not only the starch, but also other feed components. In addition, differences between sources or forms of starch may exist in terms of response to feed processing parameters (as seen in paper **II and III)**. This may confound the results as it adds extra variables between starch sources (different levels of gelatinisation) and between pelleting and extrusion processes. To avoid such confound, the same source of starch in raw or gelatinised form can be used for example in cold-pelleted diets, where the low conditioning temperatures are less likely to have a large impact on other feed components. Future studies must thus take into consideration the potentially confounding roles of feed processing and the physical form of the starch source.

More research is needed to identify the effects of different starch digestion rates on gut health and the way it affects the intestinal microbiota profile. In addition, more studies are required to understand the mechanism in which starch and protein digestion rate and site influence feed efficiency in broilers. This can be done for example by using a cold-pelleted diet based on either SBM or fish meal as the sole protein source, and either dextrose, wheat starch or bean starch as the sole glucose source in the diet. Accordingly, different glucose and amino acids absorption rates and sites can be achieved, and assessment of the nutritional regulation of these components on feed efficiency would thus be possible and less confounded by unwanted sources of variance. Finally, the effects of this dietary manipulation on the gene expression of glucose and amino acids transporters is also worth investigating.

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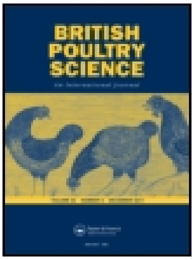
11. Papers

Paper I



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Feed processing and structural components affect starch digestion dynamics in broiler chickens

K. Itani and B. Svihus

Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway

ABSTRACT

1. A 2 × 2 factorial design was used to test the hypothesis that impaired intestinal starch digestibility is attributable to rapid passage of digesta from the gizzard to the intestine, and that, compared to steam pelleting, increasing the availability of starch through extrusion cooking may alleviate the potential negative effect of rapid digesta flow on starch utilisation.
2. Thus, 7-d-old-broiler chickens were distributed to 48 cages and given a wheat-based (WB) pelleted diet containing either coarse oat hulls (OH-Pel) or fine cellulose (Cel-Pel) until d 19 to stimulate divergent development of the gizzard. Thereafter, both groups were further subdivided and challenged with a WB diet containing cellulose in either pelleted (Cel-Pel) or extruded (Cel-Ext) form on d 20 and 22. Either excreta or intestinal contents were collected at time intervals after feeding and analysed for marker and starch.
3. OH-Pel increased gizzard size and holding capacity. No excessively high starch levels (maximum 25 g/kg) were detected in the excreta. However, 8 h feed-deprived birds given Cel-Pel and challenged with Cel-Pel exhibited higher starch excretion and showed large individual variation during the first 135 min of collection.
4. Contrary to the OH-Pel group, more digesta and starch passed to the jejunum at 1 and 2 h and ileum at 2 and 3 h after feeding for birds given Cel-Pel, resulting in lower jejunal and ileal starch digestibility.
5. Increased starch gelatinisation through extrusion processing significantly improved starch digestibility regardless of gizzard function. However, at 1, 2 and 3 h after feeding, more digesta was retained in the foregut of birds given Cel-Ext.
6. The current data showed that starch degradation rate is associated with the flow of digesta which is linked to gizzard development, and that enzymatic hydrolysis of intact starch granules may be limited with more rapid feed passage through the gut.

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Broiler; digesta flow; feed processing; starch digestion; structural component

Introduction

Starch digestibility in wheat-based (WB) pelleted diets has been observed to be low or incomplete for broiler chickens (Wiseman et al. 2000, Svihus and Hetland 2001, Abdollahi et al. 2011), with values ranging from 0.69 to 0.84 for diets containing more than 600 g/kg wheat. Poor starch digestibility has generally been attributed to several grain- or processing-related factors including the soluble fibre fraction present in wheat (Annison 1993), wheat hardness (Carré et al. 2002), resistant cell wall material (Meng et al. 2005) or a lower starch gelatinisation degree (Zimonja and Svihus 2009). Fine grinding of hard wheat (Péron et al. 2005) or the addition of fibre-degrading enzymes to wheat diets has only partially alleviated this problem (Svihus and Hetland 2001). For instance, starch digestibility in enzyme-supplemented wheat diets remained low compared to oat or barley-based diets without enzymes (Svihus 2001) and, in other studies, no relationship between grain hardness and starch digestibility was found (Rogel et al. 1987a, Amerah et al. 2007).

Enzymatic degradation of starch granules may in some cases be rate limiting; nevertheless, extrusion cooking and gelatinisation of starch has been shown to increase its susceptibility to amylase (Björck et al. 1984). Studies with broiler chickens, however, produced inconsistent results. Plavnik and Sklan (1995) observed no difference in the digestibility of starch between extruded and untreated WB diets, while Zimonja and Svihus (2009) found that, compared to cold or steam pelleting, extrusion processing significantly improved ileal starch digestibility mainly as a consequence of increased gelatinisation. These inconsistencies suggested that other, possibly bird-related, factors interfere with starch digestion of WB diets. The gizzard is the pacemaker of normal gut motility (Duke 1994) and the major site for particle size reduction and peptic proteolysis (Shires et al. 1987). Accordingly, shorter retention time in this compartment implies less physical and chemical breakdown of digesta and inadequate starch degradation along the intestinal tract (Svihus 2011b). It is well-established that gizzard activity and size are highly influenced by diet structure. Numerous workers have shown that feeding pelleted diets based on finely ground wheat reduced the grinding activity and the relative weight of the gizzard compared to diets containing coarse or large particles (Engberg et al. 2002, 2004, Amerah et al. 2009). Moreover, Svihus (2006) observed that feed intake was negatively correlated with nutrient utilisation, particularly in birds fed diets that did not stimulate gizzard activity. In addition, Svihus (2011b) reported that starch digestibility of wheat diets was correlated with the relative empty gizzard weight, as all birds with less developed

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CONTACT B. Svihus birger.svihus@nmbu.no Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

gizzards exhibited low starch digestibility. In a previous study, Svihus and Hetland (2001) indicated that an overload of wheat starch in the ileum, due to high feed intake, was the cause of reduced starch digestibility in birds given pelleted wheat diet as compared to those fed a diet with whole wheat. Accordingly, it was hypothesised that, a well-stimulated gizzard may have a regulatory effect on the flow of digesta through the digestive tract and thus on starch availability. The nutritional benefits of increasing gizzard activity using structural components in the diet is well-documented (Rogel et al. 1987b, Hetland et al. 2003, Amerah et al. 2009, Svihus 2011a), although the complete mechanism is yet to be elucidated.

Thus, the hypothesis that low intestinal starch digestibility may result from a rapid feed flow from the gizzard was tested. The gizzard of broiler chickens fed a WB diet was divergently stimulated by including either oat hulls (OH-Pel) or cellulose (Cel-Pel) powder, and digesta flow and starch digestion rate were assessed. Additionally, since extrusion as compared to pelleting generally increases starch digestibility, the birds with divergent gizzard development were fed either extruded or pelleted diets under the hypothesis that pelleted diets would have a more deleterious effect on starch digestibility than extruded diets.

Material and methods

This study was carried out in strict accordance with the laws and regulations governing experiments with live animals in Norway (the Animal Protection Act of 20 December 1974 and the Animal Protection Ordinance concerning experiments with animals of 15 January 1996).

Experimental diets and processing

Experimental diets were processed at the Centre for Feed Technology (Fôrtek), Norwegian University of Life Sciences (NMBU), Ås, Norway, and were formulated to meet or exceed Ross 308 strain recommendations (Aviagen 2014) for major nutrients (Table 1). The diets consisted of a steam-pelleted WB diets containing 50 g/kg coarse OH-Pel or fine Cel-Pel powder . In addition, the WB diet containing fine Cel-Pel powder was produced in extruded form (Cel-Ext). The above diets contained 5 g/kg titanium dioxide (TiO_2) as a digestibility marker. The wheat used was ground in a Münch hammer mill (HM 21.115, Wuppertal, Germany) fitted with a 2 mm screen prior to any processing. The mash was steam conditioned at 75°C in a double-pass pellet-press conditioner (Münch-Edelstahl, Germany) prior to pelleting (Pellet Press, Münch-Edelstahl, Germany, 1.2 t/h, 2 × 17 kW, RMP 350.100) through a 3 mm die with 42 mm thickness, at a production rate of 600 kg/h. The extruded diet was steam heated at 83°C in an extruder pre-conditioner (Bühler BCTC 10, Uzwil, Switzerland) prior to processing in a co-rotating twin-screw extruder (Bühler BCTG 62/20 D, 5 sections, 72 kW DC, Uzwil, Switzerland) fitted with 12 dies × 3 mm and with a feeder rate of 360 kg/h. A starch- and TiO_2-free fine-mash diet comprising mainly dextrose and soybean protein concentrate was produced by dry mixing the ingredients without any further processing. This diet served as a washout diet for birds prior to feed-flow measurements, to avoid an excessively long starvation period.

Table 1. Experimental diet composition, calculated and analysed nutrient content (g/kg as fed).

Ingredients	OH-Pel*	Cel-Pel/Cel-Ext*
Wheat	671.5	671.5
Fish meal (72% CP)	149	149
Soybean concentrate (68% CP)	70.1	70.1
Soybean oil	26	26
Ground limestone	12	12
L-lysine	1	1
DL-methionine	2.5	2.5
L-threonine	2.5	2.5
Mineral and vitamin premix[1]	6.4	6.4
Choline chloride	2	2
Titanium dioxide	5	5
Oat hulls (unground)	50	-
Cellulose (fine powder)[2]	-	50
Enzyme (Rovabio)[3]	1.5	1.5
Calculated nutrient content		
Metabolisable energy (MJ/kg)	12.89	12.89
Digestible lysine	13.2	13.2
Digestible methionine	6.8	6.8
Digestible threonine	10.3	10.3
Calcium (g/kg)	11	11
Available phosphorous (g/kg)	4.8	4.8
Analysed nutrient content		
Gross energy (MJ/kg)	17.0	17.0/17.1
DM (g/kg)	908	883/893
Starch (g/kg)	419	419/429
Crude protein (g/kg)	223	223/224
Starch gelatinisation[4]	318	318/975

*OH-Pel: Pelleted diet with oat hulls; Cel-Pel: Pelleted diet with cellulose; Cel-Ext: Extruded diet with cellulose;

[1] Mineral and vitamin premix provided the following per kg diet: Fe, 53 mg; Mn, 125 mg; Zn, 83 mg; Cu, 15 mg; I, 0 · 75 mg; Se, 0 · 30 mg; retinyl acetate, 5.75 mg; cholecalciferol, 0.18 mg; dl-α-tocopheryl acetate, 80 mg; menadione, 10 mg; thiamine, 6 mg; riboflavin, 26 mg; niacin, 35; calcium pantothenate, 26 mg; pyridoxine, 15 mg; cobalamin, 0.04 mg; biotin, 0.6 mg; folic acid, 5 mg.

[2] Cellulose powder: Product Sanacel 150 from CFF GmbH & Co.KG.

[3] Enzyme Rovabio Excel Ap T-Flex, Adisseo, France, provided the following per kg diet: Endo-1,4-β-xylanase: 33,000 visco units; Endo-1,3(4)-β-glucanase: 45,000 visco units; Endo-1,4-β-glucanase (cellulase) >9600 DNS units + 16 other enzyme activities obtained from a fermentation broth of *Penicillium funiculosum*.

[4] Starch gelatinisation (g/kg of total starch).

Birds, housing and management

A total of 120 1-d-old male broiler chicks were randomly allocated to four pens of 30 birds each and fed on a commercial starter pelleted diet until d 7 of age. The pens were located in an environmentally controlled, continuously lit room at the experimental farm of the NMBU, Ås, Norway. Using 2 suspended heat lamps per pen, the brooding temperature was maintained at approximately 32°C for the first 5 d and reduced to 30°C on d 7. Subsequently, room temperature was reduced by 4°C per week until an average of 22°C was reached by the end of the experiment at 22 d. The pens had wire-mesh floors covered with sheets of newspaper. On d 7, 24 birds from each pen (a total of 96 birds) were weighed and placed in pairs in 48 cages (width 50 cm× depth 35 cm× height 20 cm), so that the average weight was similar for each cage. Underweight birds were discounted. The cages had wire-mesh floor and an excreta collection tray. All birds were provided with feed and water *ad libitum* in 2 troughs attached along the front of each cage. From d 7 to d 19, the 48 cages were divided into 2 groups of 24 cages each and the birds were allocated to either OH-Pel or Cel-Pel to stimulate divergent development of the gizzard. Subsequently, to study the effect of gizzard manipulation and feed processing on digesta flow and starch utilisation, birds in each of these dietary groups were further subdivided and subjected to 2 dietary treatments on d 20 and d

22. Accordingly, the birds were challenged with a WB diet with fine cellulose in either pelleted (Cel-Pel) or extruded (Cel-Ext) form.

Excreta collection on d 20 (with feed deprivation)

In the evening of d 19, feed was withdrawn for 2 h, and then all birds (OH-Pel and Cel-Pel) were given the starch- and TiO_2-free mash diet for 8 h. This was done to ensure complete passage of previously ingested feed and thus to ensure that the digestive tract did not contain starch or TiO_2. The fine-textured mash diet was hand-mixed with water at a ratio of 3:1 (w/v) immediately preceding feeding to avoid moisture loss and to encourage prompt consumption. Thereafter, the 24 cages were divided into subgroups of 12 cages each and subjected to either 3- or 8-h feed deprivation. Subsequently, the 12 cages were further subdivided into 2 groups of six cages each and the birds were given access to either Cel-Pel or Cel-Ext for 30 min, after which feed was withdrawn and water was made freely available. Thereafter, 2 birds from each cage were separated using a cardboard to enable individual excreta collection, resulting in 12 replicate birds per combination of feeding treatments (OH-Pel or Cel-Pel), feed deprivation (3 or 8 h) and processing method (Cel-Pel or Cel-Ext). Fifteen minutes after feed removal, clean excreta trays were placed under each cage for the collection of droppings from each individual bird at 90, 135, 180, 225 and 270 min after feed access. At the end of excreta collection, the birds were given access to their respective diets (OH-Pel or Cel-Pel) until the next day. Caecal droppings, identified as brown and watery, were discarded. Excreta samples were frozen at −20°C until analysis. Due to insufficient droppings produced within each collection period, the number of birds per treatment with sufficient amount of excreta in at least 3 collection periods was only between four and six. To have an equal number of replicates per collection period, four birds per treatment were chosen at random and included in the analysis.

Excreta collection on d 21 (without feed deprivation)

After 24 h of continuous access to their respective diets, clean excreta trays were placed under each cage of the birds that were subjected to 3-h feed deprivation on d 20. After 5 h, representative samples of droppings from each cage were then collected and frozen at −20°C until analysis. This was done to measure starch digestibility and determine apparent metabolisable energy (AME) in *ad libitum*-fed, unstressed birds.

Digesta collection on d 22

In the evening of d 21, feed was withdrawn for 2 h, and then the birds were given the starch- and TiO_2-free mash diet for 8 h, and subsequently deprived of feed for 5 h. Thereafter, the 24 cages in each prior feeding treatment (OH-Pel and Cel-Pel) were divided into 2 equal groups and given access to Cel-Pel or Cel-Ext for 30 min, after which feed was withdrawn. Twenty-four birds (six per treatment) were killed each time at 1, 2 and 3 h after feed access. Despite some unavoidable minor differences in pellet appearance between the pelleted and extruded diet, no differences in feed intake were detected between the treatments (data not shown). At the time of feeding, birds were observed with minimal disturbance, and lethargic or inactive birds (3 in total) not consuming any feed were excluded from the analysis. The crop and gizzard were dissected out with care to avoid material loss and stored at −20°C until analysis. The rest of the digestive tract with its contents (excluding the colon and caeca) was placed in a zigzag pattern over an aluminium foil on a rack, snap-frozen with liquid nitrogen and stored at −20°C for later analysis. A section from the posterior jejunum including its content (5 cm from Meckel's diverticulum) was removed and stored at −80°C for later amylase activity analysis. The jejunum was defined as the segment from the end of the duodenal loop to Meckel's diverticulum and the ileum as the section from Meckel's diverticulum to the ileo–caecal junction.

Performance measurements

Body weights and feed intake per cage were recorded at 7, 14 and 21 d. Mortality was recorded as it occurred, and the 3 birds that died were weighed and feed per gain was corrected by dividing body weight gain of live plus dead birds by total feed intake.

Chemical analyses

Representative feed samples were ground in a cutting mill (Pulverisette 19, Fritsch Industriestr. 8, 55 743 Idar-Oberstein, Germany) through a 0.5 mm sieve. Gross energy was determined using an adiabatic bomb calorimeter (Parr 6400, Moline, USA) standardised with benzoic acid. Dry matter and ash content of the feed were determined after drying overnight at 105°C and after 12 h ashing at 550°C, respectively. Crude protein in the feed was determined by the Kjeldahl method. The degree of starch gelatinisation (DG; as a proportion of total starch) was measured by differential scanning calorimetry (DSC 823e Module, Mettler-Toledo, Switzerland) as described by Kraugerud and Svihus (2011) . Dry matter of the excreta, crop and gizzard content, jejunal and ileal digesta were determined after drying overnight at 105°C. Dried excreta and freeze-dried jejunal–ileal content were pulverised using a mortar and pestle for subsequent starch and TiO_2 analysis. TiO_2 content of feed, excreta, jejunal and ileal contents was determined as described by Short et al. (1996) . For starch analysis, 7–8 ml of 80% ethanol was added to each tube containing 100 ± 5 mg sample of ground feed, pulverised dried excreta or freeze-dried intestinal content. The mixture was vortexed for 5–10 s, incubated for 5 min at 80°C and centrifuged for 10 min at 3000 rpm and the supernatant containing mono-, di- and small oligosaccharides was discarded. This procedure was repeated twice. Starch content was then determined enzymatically based on the use of thermostable α-amylase and amylo-glucosidase as described by McCleary et al. (1994). Samples for amylase activity were prepared as described by Pérez de Nanclares et al. (2017) and assayed colorimetrically using amylase assay kit (Abcam-ab102523, Cambridge, UK) according to manufacturer's instructions. Activity of amylase was expressed as unit/g jejunal chyme on dry and wet basis. The amounts of digesta passing to different sections in the small intestine and starch digestibility were estimated on a dry matter basis and were calculated relative to the TiO_2 concentration.

Statistical analysis

All statistical analyses were conducted using the general linear model procedure of SAS (SAS Institute 2004). Performance parameters and excreta data (from *ad libitum*-fed birds) on d 21 were compared using Student's *t* test. Excreta data on d 20, digesta data and enzyme activity on d 22 were subjected to 2-way analysis of variance with fibre particle size and processing method as main effects. The interaction between sampling time, fibre particle and processing were not analysed statistically due to the complexity of the statistical model, and so each sampling time was analysed separately. The significance of differences between groups was determined using the Ryan–Einot–Gabriel–Welsh *F*-test. Differences were considered significant at $P < 0.05$.

Results

Excreta analysis on d 20

Although no particularly high level of starch was found in the excreta (Figure), 8 h feed-deprived birds fed Cel-Pel-containing diet during the gizzard manipulation period and challenged with the Cel-Pel exhibited higher ($P < 0.05$) starch excretion (g/kg freeze-dried excreta collected) between the first 135 and 180 min after feeding. Independent of feed-deprivation time, birds fed on the OH-Pel diet or challenged with extruded diet (Cel-Ext) showed a similar low starch excretion pattern, characterised by lower individual variation as compared to those given the Cel-Pel and challenged with Cel-Pel diet.

Performance and excreta analysis on d 21

As shown in Table 2, birds fed on diet with fine Cel-Pel tended to consume more feed ($P = 0.0945$) and were less efficient ($P < 0.001$) in feed conversion than birds given the coarse OH-Pel-containing diet. Compared to OH-Pel, Cel-Pel feeding reduced ($P < 0.001$) the AME value by 6.6% and dry matter digestibility by 7%. Moreover, although significantly different, starch levels were only 11 g/kg freeze-dried excreta, which was reflected by the nearly complete total tract starch digestibility in both groups.

Dissection results on d 22

As presented in Table 3(a–c), the content of the crop decreased with time. At 1 h following feeding, there was a trend ($P = 0.1083$) for higher DM content in the crop of birds given the extruded diet (Cel-Ext). At 2 and 3 h after feeding, birds given the Cel-Ext had significantly more

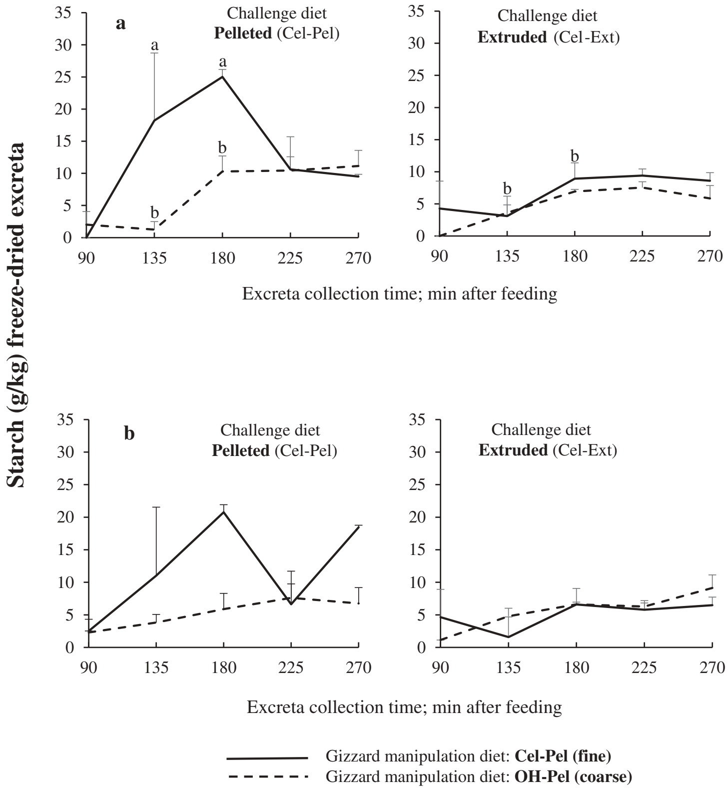

Figure. Starch content in excreta (g/kg dried excreta) collected on d 20, mean ±SEM ($n = 4$): (a) 8 h or (b) 3 h feed deprivation, followed by 30 min access to either pelleted or extruded challenge wheat-based diets. Excreta were collected 90 min after feeding and four times every 45 min thereafter. Treatment means within time with different letters are significantly different at ($P < 0.05$).

Table 2. Performance and results of excreta analysis for male broilers fed on a wheat-based pelleted diet containing either coarse or fine fibre structure.

Gizzard manipulation diet [1]	Production performance (7–21 d)			Excreta analysis[2] on d 21			
Fibre structure	Feed intake	Weight gain	Feed per gain	AME[3]	DM[3] digestibility	Starch[3] digestibility	Starch g/kg
OH-Pel* (coarse)	1261.5 ± 26.17	903.4 ± 27.79	1.40 ± 0.025	12.99 ± 0.07	0.703 ± 0.01	0.995 ± 0.00	7.69 ± 0.41
Cel-Pel* (fine)	1312.7 ± 11.90	851.6 ± 13.99	1.54 ± 0.026	12.13 ± 0.13	0.653 ± 0.01	0.988 ± 0.00	11.34 ± 0.82
P values	0.0945	0.1147	<0.001	<0.001	<0.001	<0.001	0.0013

[1] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls (OH-Pel) or fine cellulose (Cel-Pel).
[2] After 24 h of open access to feed, clean excreta trays were placed under each cage for 5 h, then representative samples of droppings from each cage were collected, oven dried and analysed.
[3] Apparent metabolisable energy (AME) MJ/kg DM, total tract dry matter (DM) and starch digestibility were calculated using marker techniques.
*Values are means ± SEM (n = 12 replicate cages of 2 birds each) and are significantly different at ($P < 0.05$).

material in the crop than the birds given the pelleted diet (Cel-Pel). At 1 and 2 h after feeding, a higher ($P < 0.05$) dry matter content was found in the gizzard of birds given Cel-Ext. As expected, OH-Pel had a large ($P < 0.0001$) stimulating effect on gizzard development and holding capacity, expressed as relative empty weight and dry matter content, respectively. There was an increase in the amount of dry matter flowing to the jejunum at 1 h ($P = 0.001$) and 2 h ($P = 0.0236$) and to the ileum at 2 h ($P = 0.0568$) and 3 h ($P = 0.0883$) for birds given the diet containing Cel-Pel. The pattern of starch flow closely followed that of dry matter at the jejunal and ileal levels. Accordingly, jejunal starch concentration was lower in birds fed on diet with coarse structure (OH-Pel) at 2 and 3 h ($P = 0.0007$ and $P = 0.0998$) respectively. A significant ($P = 0.0295$) interaction was observed at 1 h between fibre structure and processing method on starch content in the jejunum. As a result, birds given the Cel-Pel during gizzard manipulation period had higher concentrations of starch in the jejunum when challenged with pelleted diet (Cel-Pel). Ileal starch concentrations were lower at 2 and 3 h ($P = 0.0089$ and $P = 0.0223$), respectively, for OH-Pel group. This resulted in higher starch digestibility at both jejunal (at 1 h, $P = 0.0447$ and 2 h, $P = 0.0004$) and ileal (at 2 h, $P = 0.0101$) level. The effect of fibre structure on ileal starch digestibility was less obvious ($P = 0.0957$) at 3 h after feeding, even though starch concentrations were significantly lower in the OH-Pel group. Additionally, a significant main effect of feed processing on digesta flow into the intestine was observed. Accordingly, lower content of digesta entered the jejunum and ileum at 1 h ($P = 0.0037$ and $P = 0.0228$, respectively) and the ileum ($P = 0.0438$) at 3 h for birds receiving the extruded diet (Cel-Ext). Starch content (g/kg in freeze-dried jejunal and ileal contents) was consistently and significantly lower for birds challenged with Cel-Ext as compared with those challenged with the Cel-Pel at all killing times. Consequently, extrusion resulted in significantly higher starch digestibility and tended ($P = 0.1073$) to alleviate the negative effect of lack of OH-Pel (i.e. gizzard stimulation) on ileal digestibility.

Amylase activity

As shown in Table 4, jejunal amylase activity was not affected by feed processing method ($P > 0.1$). However, there was a tendency ($P = 0.0963$) for a higher amylase activity in birds given the OH-Pel as compared to Cel-Pel. When expressed as unit per gram of dry chyme, the tendency was higher but did not reach significance ($P = 0.0797$).

Discussion

The current experiment demonstrated the rapid passage of digesta from the gizzard into the intestine when the gizzard was insufficiently stimulated. In addition, compared to pelleting, starch digestibility in the extruded diet seemed to be less affected by gizzard function. This initially supported the hypothesis for a negative consequence of rapid passage of digesta on more digestion-resistant components, i.e. in pelleted diets. Before incorporation, wheat was finely ground (2 mm screen size) to avoid any confounding effect of coarse grain grinding on gizzard stimulation (Svihus 2011a) or grain hardness on starch accessibility (Péron et al. 2005). In addition, diets were supplied with fibre-degrading enzymes to eliminate any potential effect of the soluble fibre fraction in wheat on digesta viscosity (Choct et al. 1996). The ability of the avian gizzard to exhibit rapid phenotypic responses to dietary stimuli was previously demonstrated by Starck and Rahmaan (2003). Thus, the stimulatory effect of OH-Pel on gizzard development in this experiment was expected and is in line with previous reports (Hetland et al. 2003, Sacranie et al. 2012).

Excreta analysis showed no sign of high starch levels (maximum 25 g/kg) being excreted independently of the lengths of feed deprivation used in this experiment. Comparable levels of starch in the excreta were also detected by Svihus and Hetland (2001), although no feed deprivation was used. Accordingly, they reported values ranging from 20 to 47 g/kg for a cellulose-diluted (10%) or undiluted pelleted WB diet, respectively. Similarly, with unprocessed mash diets, cereal grains had an undigested starch fraction of between 20 and 60 g/kg freeze-dried excreta (Weurding et al. 2001). It is worth mentioning that the individual variation and starch levels were higher at the beginning of excreta collection (135 min) particularly for birds with smaller gizzards and challenged with pelleted diet after 8-h feed deprivation. This suggested that the combination of a rapid passage of digesta into the intestine, due to inadequate stimulation of gizzard function, and insufficient degradation of starch may be the cause for the higher amount of starch lost in excreta. Nevertheless, the magnitude was lower than expected. The very small amount of starch in the excreta indicated that starch digestibility was very high or nearly complete (data not shown). It should be noted that a fraction of starch may be lost in the lower digestive tract due to microbial fermentation in the caeca (Svihus et al. 2013) . Thus, total tract digestibility values may in some cases (Marron et al. 2001) give an inaccurate picture of starch digestibility (Svihus and Hetland 2001). Therefore, analysing ileal content allowed for more precise assessment on the fate of starch and confirmed that starch

Table 3. The influence of fibre structure and processing method on the weight of crop and gizzard contents, relative weight of empty gizzard, weight of digesta (expressed on a DM basis) passing to the jejunum and ileum, starch content in freeze-dried jejunal and ileal contents and starch digestibility in broilers killed at different times.

a. From 7–21 d	At 22 d	Killed at 1 h after feeding[1]								
Gizzard manipulation diet[2]	Challenge diet[2]	Crop	Gizzard		Jejunum			Ileum		
Fibre structure	Processing method	DM g	DM g	Rel. w. g/kg	Digesta[3]	Starch g/kg	Starch digestibility	Digesta[3]	Starch g/kg	Starch digestibility
OH-Pel (coarse)	Cel-Ext	14.8	2.0	15.2	2.0	23.4 c	0.971	0.8	7.5	0.989
Cel-Pel (fine)	Cel-Ext	15.8	1.1	9.5	2.7	40.8 c	0.952	0.7	9.5	0.987
OH-Pel (coarse)	Cel-Pel	12.2	1.4	17.1	2.6	129.2 b	0.802	1.3	80.1	0.861
Cel-Pel (fine)	Cel-Pel	10.6	0.6	11.0	3.3	224.1 a	0.690	1.5	99.9	0.849
√MSE[4]		5.49	0.62	2.82	0.43	39.40	0.082	0.66	45.19	0.064
Fibre										
Coarse		13.5	1.7 a	16.2 a	2.3 b	76.3	0.887 a	1.1	40.5	0.925
Fine		13.0	0.8 b	10.3 b	3.0 a	140.8	0.821 b	1.1	54.8	0.918
Processing										
Extrusion		15.3	1.6 a	12.6	2.3 b	31.3	0.962 a	0.8 b	8.5 b	0.988 a
Pelleting		11.4	1.0 b	14.0	3.0 a	176.6	0.746 b	1.4 a	90.0 a	0.855 b
P value										
Fibre		0.8947	0.0030	<0.0001	0.0010	0.0029	0.0447	0.8242	0.5859	0.7907
Processing		0.1083	0.0368	0.1694	0.0037	<0.0001	<0.0001	0.0228	0.0008	<0.0001
Fibre × processing		0.5760	0.8002	0.8792	0.9927	0.0295	0.1276	0.5619	0.6624	0.7377

b. From 7–21 d	At 22 d	Killed at 2 h after feeding[1]								
Gizzard manipulation diet[2]	Challenge diet[2]	Crop	Gizzard		Jejunum			Ileum		
Fibre structure	Processing method	DM g	DM g	Rel. w. g/kg	Digesta[3]	Starch g/kg	Starch digestibility	Digesta[3]	Starch g/kg	Starch digestibility
OH-Pel (coarse)	Cel-Ext	13.7	2.1	14.8	1.8	13.2	0.986	3.3	6.4 b	0.995
Cel-Pel (fine)	Cel-Ext	11.4	0.8	9.9	2.7	53.9	0.928	3.9	18.6 b	0.985
OH-Pel (coarse)	Cel-Pel	7.4	1.8	15.3	2.1	60.5	0.931	2.3	12.2 b	0.987
Cel-Pel (fine)	Cel-Pel	7.5	0.1	8.8	3.0	101.5	0.867	3.9	62.6 a	0.947
√MSE[4]	4.84	0.51	2.15	0.88	24.31	0.037	1.29	25.71	0.023	
Fibre										
Coarse		10.8	2.0 a	15.1 a	1.9 b	34.7 b	0.959 a	2.8	9.0 b	0.991 a
Fine		9.4	0.5 b	9.4 b	2.9 a	77.7 a	0.898 b	3.9	40.6 a	0.966 b
Processing										
Extrusion		12.6 a	1.4 a	12.4	2.3	33.5 b	0.957 a	3.6	12.5 b	0.990 a
Pelleting		7.5 b	0.9 b	12.1	2.6	82.8 a	0.899 b	3.1	39.7 a	0.967 b
P value										
Fibre		0.5917	<0.0001	<0.0001	0.0236	0.0007	0.0004	0.0568	0.0089	0.0101
Processing		0.0208	0.0462	0.7235	0.4394	0.0001	0.0007	0.3386	0.0315	<0.0001
Fibre × processing		0.5754	0.3390	0.3828	0.9822	0.9914	0.8401	0.4181	0.0918	0.1073

c. From 7–21 d	At 22 d	Killed at 3 h after feeding[1]								
Gizzard manipulation diet[2]	Challenge diet[2]	Crop	Gizzard		Jejunum			Ileum		
Fibre structure	Processing method	DM g	DM g	Rel. w. g/kg	Digesta[3]	Starch g/kg	Starch digestibility	Digesta[3]	Starch g/kg	Starch digestibility
OH-Pel (coarse)	Cel-Ext	5.6	1.4	18.4	2.6	30.8	0.968	3.7	13.7	0.989
Cel-Pel (fine)	Cel-Ext	2.5	0.4	10.9	2.9	39.9	0.957	3.9	19.5	0.985
OH-Pel (coarse)	Cel-Pel	2.6	1.4	16.8	2.7	81.9	0.920	4.1	20.6	0.981
Cel-Pel (fine)	Cel-Pel	1.4	0.1	10.4	3.9	111.6	0.888	5.9	48.1	0.964
	√MSE[4]	2.09	0.59	2.89	1.06	26.14	0.036	1.31	16.02	0.013
Fibre										
Coarse		4.1 a	1.4 a	17.6 a	2.7	56.3	0.944	3.8	17.2 b	0.985

(*Continued*)

Table 3. (Continued).

c. From 7–21 d

Gizzard manipulation diet^2	Challenge diet^2	Crop	Gizzard		Jejunum			Ileum		
Fibre structure	Processing method	DM g	DM g	Rel. w. g/kg	Digesta^3	Starch g/kg	Starch digestibility	Digesta^3	Starch g/kg	Starch digestibility
Fine		2.0 b	0.3 b	10.7 b	3.2	75.8	0.923	4.8	32.5 a	0.975
Processing										
Extrusion		4.1 a	0.9	14.6	2.7	34.9 b	0.963 a	3.8 b	16.6 b	0.987 a
Pelleting		2.0 b	0.8	13.9	3.2	95.4 a	0.904 b	5.0 a	33.1 a	0.973 b
P value										
Fibre		0.0198	0.0002	<0.0001	0.1405	0.0998	0.1402	0.0883	0.0223	0.0957
Processing		0.0268	0.5726	0.4043	0.2674	<0.0001	0.0006	0.0438	0.0158	0.0218
Fibre × processing		0.2953	0.5421	0.6731	0.3279	0.3702	0.4607	0.1566	0.1219	0.3103

Column groups: At 22 d (Gizzard manipulation diet, Challenge diet); Killed at 3 h after feeding^1 (Crop, Gizzard, Jejunum, Ileum).

[1] Values are means of six replicate birds.

[2] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls (OH-Pel) or fine cellulose (Cel-Pel); Challenge diet: WB diet with fine cellulose in extruded (Cel-Ext) or pelleted (Cel-Pel) form.

[3] The weight of digesta passing into the jejunum and ileum was estimated on a DM basis and calculated relative to the TiO$_2$ concentration in freeze-dried digesta.

[4] $\sqrt{}$MSE: square root of means square error in the analysis of variance.

[a,b,c] Means within column followed by different letters are significantly different at ($P < 0.05$).

was highly digestible, even in stress conditions such as feed deprivation.

Two main observations can be drawn from the dissection results: First, differences in digesta flow and the amount of starch recovered in the small intestine were likely influenced by the rate at which feed was leaving the gizzard. Independent of the processing method, digesta passed into the intestine faster for birds with smaller gizzards. Accordingly, more starch reached the jejunum or ileum, which caused a reduction in starch digestibility in the respective intestinal segment. On the contrary, due to OH-Pel inclusion, larger gizzards were able to hinder the fast flow of digesta into the jejunum at 1 and 2 h and into the ileum at 2 and 3 h after feeding. The current results are in line with recent findings. Already, 1 h after feeding, Sacranie et al. (2017) showed higher ($P < 0.05$) load of DM and starch in the small intestine of 16 h-starved birds, adapted to, and re-fed, a diet with fine cellulose as compared with coarse OH-Pel. Using whole wheat as gizzard-stimulating components, Svihus et al. (2010) reported that the jejunum and ileum of birds killed 1 h after re-feeding contained less ($P = 0.01$) DM for the whole-wheat diet compared with the ground wheat diet. This was accompanied with a concomitant reduction ($P < 0.001$) in the ileal concentration of starch and improvement ($P < 0.001$) in total tract starch digestibility for the whole-wheat diet.

In the current experiment, the challenge diets contained the same source of fibre (fine cellulose powder) and thus only differed in the way they were processed (pelleted vs. extruded). In the aforementioned studies, the challenge diets given to feed-deprived birds contained different structural components, as already mentioned. Using the same source of fibre, this experiment eliminated the potential confound of coarse or fine structure on digesta passage and clearly demonstrated the ability of a well-functioning gizzard in modulating the flow of feed, even when lacking structural components. The above observations emphasised the importance of the gizzard as a feed-flow regulator (Svihus 2014, Classen et al. 2016, Sacranie et al. 2017) and validated the hypothesis that the gizzard may be the key site for prevention of starch overload in the digestive tract (Svihus and Hetland 2001).

Second, the more vigorous conditions in the extrusion processing are generally sufficient to cause complete disruption of starch granule structure (Skoch et al. 1983, Svihus et al. 2005), which is expected to increase the susceptibility of starch to enzymatic hydrolysis (Björck et al. 1984, Sun et al. 2006). These results are in accordance with those reported by Zimonja and Svihus (2009), where higher gelatinisation of starch in the extrusion processing significantly increased starch digestibility in wheat diets. However, it was observed during dissection that the content of the crop and gizzard differed in physical appearance between the extruded and pelleted diets. Crop and gizzard digesta appeared lumpy with intact and swollen pellets for the extruded diet, while it was watery with no apparent intact pellets for the pelleted diet. Hilton et al. (1981) reported similar observations and attributed this to the higher water stability of the extruded diets which increases its retention time in the upper gut compartments. This is consistent with the current results, where higher DM was found in the crop and gizzard for birds given the extruded diets at least in the first 2 h after feeding. With such characteristics, extruded diets tend to

Table 4. Amylase activity in the jejunum of 22-d-old broilers as influenced by fibre structure and processing method.

From 7–21 d	At 22 d		Amylase activity [1]	
Gizzard manipulationdiet[2]	Challenge diet[2]		Wet chyme	Dry chyme
Fibre structure	Processing method		Unit/g	Unit/g
OH-Pel (coarse)	Cel-Ext		118.6	563.0
Cel-Pel (fine)	Cel-Ext		90.6	415.8
OH-Pel (coarse)	Cel-Pel		99.3	447.8
Cel-Pel (fine)	Cel-Pel		73.4	337.6
		√MSE [3]	35.01	159.14
Fibre				
Coarse			108.9	505.4
Fine			82.8	380.0
Processing				
Extrusion			103.3	482.5
Pelleting			86.3	392.7
P value				
Fibre			0.0963	0.0797
Processing			0.2501	0.1870
Fibre × processing			0.9483	0.7914

[1] Values are means of six replicate birds killed 2 h after feeding.

[2] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls OH-Pel or fine cellulose Cel-Pel; Challenge diet: WB diet with fine cellulose in extruded Cel-Ext or pelleted Cel-Pel form.

[3] √MSE: square root of means square error in the analysis of variance.

have a slower passage rate than the pelleted diet, and interaction between feed processing, feed flow and starch availability may exist. The longer time required to moisturise the extruded feed in the upper gut could be a potential confounding factor affecting starch availability. An improved nutrient digestibility and feed efficiency have been associated with slower digesta transit time caused by longer retention of the feed in the crop (Svihus 2014, Classen et al. 2016) and gizzard (Sacranie et al. 2012). Therefore, care must be taken before drawing firm conclusions regarding the cause of the high digestibility of starch in the extruded diet.

A combination of factors in this experiment may have contributed to the high starch digestibility even in pelleted diets, such as the fine grinding of the wheat and enzyme addition. However, the latter variables were held constant for both groups except for gizzard stimulation. Moreover, contrary to the findings of Hetland et al. (2003), no difference in amylase activity was observed which could explain the high starch digestibility in all treatments. Although starch excretion/digestibility was statistically different between treatments, the difference was smaller than expected. As a result, birds fed on a diet without structure and challenged with a pelleted diet were able to cope with the stress and surprisingly exhibited high starch digestibility. In this case, improved gizzard function does not solely explain this high starch availability and thus, other mechanisms must be involved. Unlike mammals, vigorous gut refluxes are normal in birds (Duke 1997), and as Basha and Duke (1999) stated, intestinal refluxes are uniquely avian. Sacranie et al. (2007) found that intestinal reflux, or the retrograde movement of digesta, occurs throughout the digestive tract of both fasted and fed chickens. Reflux, therefore, serves to re-expose intestinal digesta to gastric secretions, thereby extending the digestive and absorptive processes to compensate for the lack of food and short intestinal segments (Duke 1997; Sacranie et al. 2005). The small amount of starch excreted, despite higher starch content in ileal digesta, seems to support this postulation.

In conclusion, the current data showed that the rapid passage of digesta to the small intestine resulted in reduced starch digestibility, particularly with lower degree of starch gelatinisation. This suggested that starch degradation rate is associated with the flow of digesta which may be linked to gizzard development, and that enzymatic accessibility of intact starch granules can be limiting with more rapid feed passage through the gut.

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Paper II

VARYING RATIO OF STARCH TO FAT IN BROILER DIET: 1. EFFECTS ON NUTRIENT DIGESTIBILITY AND PRODUCTION PERFORMANCE.

K. Itani [a†], S. Granstad [b†], L.T. Mydland [a], M. Kaldhusdal [b] & B. Svihus [a*]
[a] Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway; [b] Norwegian Veterinary Institute, Oslo, Norway.

[†]Shared first authorship.

[*]Corresponding author:
birger.svihus@nmbu.no

ABSTRACT

1. The hypothesis of this experiment was that a diet with a high ratio of starch to fat (**HRSF**) may impair nutrient digestibility and growth performance as compared to a diet with a low ratio of starch to fat (**LRSF**). From d 10 to 29, broilers in 12 replicate pens were given isocaloric and isonitrogenous diets with either a **HRSF** or **LRSF**, by replacing the isolated wheat starch (**WS**) in one diet by a mixture of rapeseed oil and sand in the other diet. At d 17, a 10-fold dose of live vaccine strains of *Eimeria* species was administered in the drinking water to predispose the birds to intestinal proliferation of *Clostridium perfringens*. Ileal samples were collected on d 16 and 29.
2. Weight gain did not differ among the treatments, however birds fed **LRSF** were less efficient in feed conversion as compared to those fed **HRSF**.
3. Ileal starch digestibility tended to be higher at d 16 and was higher at d 29 for the **HRSF** group, while ileal energy digestibility was not affected by the treatments.
4. The **HRSF** did not induce an overload of starch in the ileum. Accordingly, ileal starch digestibility was improved with increasing dietary starch level from 23 to 45 %, demonstrating the high capacity of the broiler chicken to digest high levels of starch even under challenging conditions.
5. The inadvertently higher extent of starch gelatinisation and the use of isolated WS in the **HRSF**, as well as possible lipid-amylose interactions in the **LRSF** may have caused the increased starch digestibility in the **HRSF**. Therefore, our data cannot be used to reject the hypothesis that **HRSF** may impair digestibility and production performance. Further work is required to clarify this research question, taking into consideration the potentially confounding roles of feed processing and physical form of starch sources.

Introduction

Young broilers appear to be efficient at utilising starch as their main energy source (Thomas et al. 2008). This ability is presumably due to sufficient amylase secretion (Svihus 2014), high activity levels of disaccharidases shortly after hatching (Chotinsky et al. 2001) and a highly adaptive intestinal mechanism for glucose uptake (Suvarna et al. 2005). Nevertheless, starch digestibility has been observed to be low in broilers given wheat-based pelleted diets with values ranging from 0.76 to 0.93 (Svihus 2001, Svihus et al. 2010, Abdollahi et al. 2011). Svihus and Hetland (2001) evaluated starch digestibility in birds fed identical wheat diets that were either pelleted or offered as mash. Feeding the diet in pelleted form resulted in an increase in feed intake which was associated with higher concentration of wheat-starch in ileal chyme and thus poorer starch digestibility. This observation made the authors propose that an overload of WS might be the cause of poor digestibility in some broilers.

Increased amounts of undigested nutrients in the digestive tract may favour intestinal fermentation by stimulating undesirable microbial growth that could induce enteric disorders (Choct et al. 1999, Annett et al. 2002). Corroborating this, Engberg et al. (2004) found a tendency for increased ileal and caecal numbers of *C. perfringens* due to the presence of more starch and other fermentable nutrients in the small intestine of broilers fed on a pelleted wheat diet. *Eimeria* infections is another factor that may lead to microbial and intestinal dysfunctions (Yun et al. 2000, Hauck 2017), and consequently increase the vulnerability of the broiler intestine to other types of intestinal insults and imbalances.

Starch is the major energy-supplying source in broiler diets, but when prices are favourable it may be preferred to replace starch with fat in the diet. However due to the rising prices of cereal grains, the use of grain-replacing, unconventional feedstuffs is increasing, and so more fat is added to increase dietary energy content. Accordingly, the effects of different ratios of starch: fat on the performance of broilers fed isocaloric and isonitrogenous diets have been investigated and produced inconsistent results. Veldkamp et al., (2017a, 2017b) for instance, reported an improvement in feed conversion ratio (FCR) and growth performance with higher ratio of starch: fat. Malheiros et al. (2004) on the other hand, reported slightly better FCR with lower ratio of starch: fat, whereas Baéza et al. (2015) found that performance parameters were not affected by the varying ratios of starch: fat.

Thus, the hypothesis was tested that a diet with high ratio of starch: fat will result in lower intestinal starch digestibility and high concentrations of undigested starch in the posterior small intestine, which in turn may impair production performance and promote a dysfunctional microbiota and suboptimal intestinal health. The present paper focuses on nutrient digestibility and production performance, while the effects on intestinal health and microbiota will be discussed in an accompanying paper (Granstad et al., manuscript in preparation).

Materials and methods

Experimental diets and processing

Experimental diets (**Table 1**) were processed at the Centre for Feed Technology (Fôrtek), Norwegian University of Life Sciences, Ås, Norway, and were formulated to meet or exceed Ross 308 strain recommendations for major nutrients (Aviagen 2014). The diets contained 5 g/kg titanium dioxide as a digestibility marker. The wheat and soybean meal (SBM) were ground to pass through a 3-mm sieve in a hammer mill (Münch-Edelstahl, Wuppertal, Germany licensed by Bliss, USA, 18.5kW, 3000 RPM) before being mixed with other ingredients. The mash was steam-conditioned in a double pass pellet-press conditioner (Münch-Edelstahl, Wuppertal, Germany) and then pelleted using a pellet press (Münch-Edelstahl, Wuppertal, Germany, 1.2 t/h, 2×17 kW, RMP 350) equipped with a 60-mm-thick die with 5-mm diameter die openings. Conditioning temperature and production rates were 71°C and 700 kg/h for the diet with a high ratio of starch to fat (**HRSF**), and 81°C and 800 kg/h for the diet with a low ratio of starch to fat (**LRSF**). Specific energy consumption values were 45.7 and 18.5 kWh/t, and motor load was 52 and 24 amperes for the diet with a **HRSF** and **LRSF,** respectively. Despite the reduced conditioning temperature, post-pelleting temperatures were 95°C in the diet with a **HRSF** compared to 81.9°C for the diet with a **LRSF**, measured by collecting a sample of hot pellets from immediately below the pellet press into an insulated box fitted with a thermometer. The extent of starch gelatinisation was almost 7.3-fold higher with a **HRSF** compared to a **LRSF** (**Table 1**).

Birds and housing

The animal experiment was approved by the national animal research unit (Forsøksdyrforvaltningen) at the Norwegian Food Safety Authority (FOTS id 8824). A total of 1920 one-day old mixed-sex Ross 308 broiler chickens obtained from a commercial hatchery (Nortura Samvirkekylling, Våler, Norway) were placed in 24 floor pens of 5.6 m^2 on wood shavings (80 birds per pen). A temperature of 33°C was maintained during the first week and thereafter decreased by 3- 4°C weekly until the room temperature reached 21°C. Water and feed were given *ad libitum*. The chickens were exposed to light during 23 hours a day on the first two days. For the rest of the experimental period, the chickens were exposed to light during 16 hours a day, interrupted by two 4-hour periods of darkness. After nine days on a commercial starter diet, the diets with a HRSF and LRSF were randomly allocated to 12 pens each. As mentioned above, the diets were pelleted, as this feed form allows for a high feed intake.

Eimeria challenge

A 10-fold dose of Paracox-5 vet (MSD Animal Health, Bergen, Norway) containing live, sporulated oocysts from 5 attenuated strains of *Eimeria spp*. (one strain of *E. acervulina*, one strain of *E. mitis*, two strains of *E. maxima*, and one strain of *E. tenella*) was administered into the drinking water of all birds on day 17 post hatch.

Production performance measurements

The birds and the feed intake were weighed on a pen basis on d 10, 15, 24 and 28. Performance data was adjusted for mortality, which was recorded daily.

Sample collection

At d 16 and 29, two birds per pen were killed by a cranial blow followed by cervical dislocation. Then, the small intestine with content was removed and placed in a zic-zac pattern over an aluminium foil on a rack, snap-frozen with liquid nitrogen and stored at −20°C for later analysis. A section from the posterior jejunum with content (5 cm anterior to Meckel's diverticulum) was later removed and stored at −80°C for later enzyme activity analysis. The jejunum was defined as the segment from the end of the duodenal loop to Meckel's diverticulum, and the ileum as the section from Meckel's diverticulum to the ileo-caecal junction.

Chemical analyses

Representative feed samples were ground on a cutting mill (Pulverisette 19, Fritsch Industriestr. 8, 55743 Idar-Oberstein, Germany) through a 0.5 mm sieve. Dry matter (**DM**) and ash content of the feed and ileal samples were determined after drying overnight at 105°C and after 12 h ashing at 550°C, respectively. Gross energy was determined using an adiabatic bomb calorimeter (Parr 6400, Moline, USA) standardized with benzoic acid. Nitrogen content was determined by the Dumas method using a Vario El Cube (Elementar Analysensysteme GmbH, Hanau, Germany 2016). Dried ileal contents were pulverized using a mortar and pestle for subsequent starch, ether extract, gross energy and titanium dioxide analysis. TiO_2 content of feed and ileal contents was determined as described by Short et al. (1996). Ether extract was determined after extraction with 80% petroleum ether and 20% acetone in an Accelerated Solvent Extractor from Dionex (ASE200; Sunnyvale, CA, USA). Starch content in the diets was determined enzymatically based on the use of thermostable α-amylase and amylo-glucosidase (McCleary et al. 1994). Starch content in freeze-dried ileal samples was determined as described above after extraction with 80% ethanol (2x) to remove free sugars and oligosaccharides. Amylase activity in the jejunal chyme was assayed colorimetrically using amylase assay kit (Abcam- ab102523, Cambridge, UK) according to manufacturer's instructions. Samples for amylase activity were prepared as described by Pérez de Nanclares et al. (2017) and results were expressed as unit/g of wet chyme. The degree of starch gelatinisation (DG) (as a proportion of total starch) was measured by differential scanning calorimetry (DSC 823e Module, Mettler-Toledo, Switzerland) as described by Kraugerud and Svihus (2011).

Calculations

The apparent ileal digestibility coefficients of starch, fat and energy were calculated using the following formula:

$$\text{Ileal digestibility coefficient}= \frac{\left(\frac{Nut}{Ti}\right) diet - \left(\frac{Nut}{Ti}\right) ileum}{\left(\frac{Nut}{Ti}\right) diet}$$

Where $\left(\frac{Nut}{Ti}\right) diet$= the ratio of nutrient and TiO_2 in the diet and $\left(\frac{Nut}{Ti}\right) ileum$= the ratio of nutrient and TiO_2 in the ileal digesta.

Statistical analysis

Statistical analyses were carried out using the statistical software R version 2.3.2. All data sets were tested for normality using the Shapiro Wilk test and were compared using Student's t-test after confirming that the data were normally distributed. A non-normal distribution of nutrient content in ileal digesta and nutrient digestibility precluded the use of a parametric statistical test and hence were compared using the two-way Wilcoxon test (non-parametric). Differences were considered significant at $P < 0.05$ and results were expressed as means ± standard error. Pen was used as the experimental unit for all data.

Results:

Production performance:

As shown in **Table 2**, from 10 to 15 d, no significant differences in feed intake (**FI**), body weight gain (**BWG**) or feed conversion ratio (**FCR**) were observed between dietary treatments. From 15 to 24 d, birds in both groups had similar FI, but those in the **HRSF** group gained more weight ($P < 0.05$) and consequently had a superior FCR ($P < 0.001$). From 24 to 28 d, birds given the diet with a **LRSF** consumed significantly more feed than those fed the diet with a **HRSF**, and still gained similar weight, resulting in higher FCR ($P < 0.01$). Overall, no difference in BWG was found ($P > 0.05$) between treatments. Birds in the **LRSF** group consumed more feed ($P = 0.0210$) and thus were less efficient in feed conversion ($P < 0.001$) as compared to the **HRSF** group.

Ileal digestibility coefficients and amylase activity

The freeze-dried weight of ileal digesta was significantly higher with a LRSF in the diet (containing 16.26% sand), resulting in lower ileal DM digestibility compared to those fed on the diet with a HRSF (data not shown). As shown in **Table 3**, starch content in the ileum was significantly influenced by diet composition. Starch digestibility tended to be higher at d 16 ($P = 0.0832$), and was higher ($P < 0.05$) at d 29 in birds fed the diet with a **HRSF**. The apparent fat digestibility was significantly higher with a **LRSF** in the diet at both ages, while the apparent energy digestibility was not different ($P > 0.05$) between the treatments. Though not significant, there was a large numerical difference (50 to 45%) in amylase activity between the treatments. Thus, there was a trend ($P = 0.1112$) and a tendency ($P = 0.0831$) for higher (50 - 45%) amylase activity in the jejunum of birds fed on the diet with **HRSF** at 16 and 29 d, respectively.

Discussion

The current experiment demonstrated the large flexibility of broilers in terms of capacity to thrive on diets containing large variations in the ratios of starch: fat and high level of sand as an inert filler. Compared to a **LRSF**, feeding a diet with a **HRSF** was expected to cause a reduction in starch digestibility, which in turn might impair production performance and intestinal health. However, a **HRSF** in the diet was associated with improved rather than impaired starch digestibility and production performance. Poor starch-digestibility in wheat diets has been attributed to several different factors, including the soluble fibre-fraction in wheat (Annison 1993), wheat hardness (Carré et al. 2002), resistant cell wall material (Meng et al. 2005), and a lower starch gelatinisation degree (Zimonja and Svihus 2009). Overload of WS in the digestive tract has also been suggested to cause poor starch digestibility (Svihus and Hetland 2001). In contrast to Svihus and Hetland (2001) who reported an average starch content of 222 g/kg ileal DM in a group of broilers exhibiting poor starch digestibility, maximum starch concentration in freeze-dried ileal content in our experiment did not exceed 80 g/kg which is similar to what Svihus and Hetland (2001) found in a group of broilers exhibiting higher starch digestibility. The wheat in the current experiment was finely ground, and the diets were supplied with fibre-degrading enzymes to eliminate any potential effect of the cell wall or insoluble fibre fraction on nutrient encapsulation and digesta viscosity.

The surprisingly higher starch digestibility with a HRSF, and the unanticipated lower starch digestibility with a LRSF may be explained by inadvertent confounding factors, not least the observed higher extent of gelatinisation (by 7.3-fold) in the HRSF compared with the LRSF. A higher starch gelatinisation is known to increase the susceptibility of starch to enzymatic hydrolysis (Mollah et al. 1983, Holm et al. 1988, Ankrah et al. 1999, Zimonja and Svihus 2009). The 14% difference in hot pellet temperature between the diets clearly indicates that, like soy oil (Cutlip et al. 2008), rapeseed oil in the diet with a LRSF had a lubricating effect, and as a result, decreased friction in the pellet die, which is the only source of heat at that point. This is supported by the pellet mill throughput and energy consumption data. In contrast, the very low oil content with a HRSF led to increased friction in the die, i.e. higher pellet temperature, and consequently higher degree of starch gelatinisation (Thomas et al. 1998). It is also important to note that, despite the lower starch digestibility in the diet with a LRSF at d 16, the average concentration of undigested starch in ileal contents was still low (58 g/kg). It has been shown that starch gelatinisation can be modified, delayed or inhibited by the presence of lipids (Larsson 1980, Eliasson et al. 1981, Lund and Lorenz 1984). Also, lipids are known to form inclusion compounds with amylose (Putseys et al. 2010, López et al. 2012) during processing or in the intestine (Holm et al. 1983). In fact, due to its hydrophobic nature, fat may interfere with the hydration of feed components, for example by coating starch granules and limiting steam penetration (Zimonja et al. 2007), thus repressing swelling and solubilisation (Eliasson et al. 1981, Svihus et al. 2005) and reducing the rate of starch hydrolysis (Tufvesson et al. 2001). Fat digestibility improved with age and was significantly higher with increasing fat inclusion. Although not evaluated, this may be due to the increase in lipase activity (Krogdahl and Sell 1989). At d 29 in the LRSF group, the proportion of fat remaining in the intestine was lower, i.e. less fat was present to complex with starch (Crowe et al. 2000), which might make more starch available for amylase digestion. The concomitant improvement in starch digestibility with age in the LRSF group (0.894 at d 16 vs 0.950 at d 29) is in line with this speculation, especially that amylase activity was similar at both ages. Also, it may indicate that a higher degree of starch gelatinisation was required for younger birds and that older birds would also benefit from it. Supporting this postulation is the higher starch digestibility at d 16 in the HRSF group despite higher starch content in their diet (7.3-fold higher gelatinisation than the LRSF).

Another plausible cause for the high starch digestibility with a HRSF was the use of the isolated wheat-starch. This starch source was added to increase starch content in the diet, which was hypothesised to cause high concentrations of starch in the lower tract. Evidently, isolated wheat-starch was not challenging enough for the birds, suggesting a fast rate of degradation in the upper intestinal tract. Amylase results showed a trend characterised by an increase or decrease in amylase activity depending on the amount of substrate in the digesta, as demonstrated before (Karasov and Hume 1997). This physiological adaptation (Murugesan et al. 2014) may thus, at least partly, explain the high capacity of the birds to digest high levels of starch in the diet.

The lower apparent fat digestibility with HRSF may be attributed to the low content of dietary fat (14.2 g/kg) and a relatively higher contribution of endogenous losses such as bile acids esters, cholesterol or structural lipids from desquamated cells (Jørgensen et al. 1993). It may therefore be that broilers have a large capacity to utilise fat, however, due to the very low-fat content in the HRSF, fat digestibility results in this group may be unreliable.

The two diets differed significantly with regard to overall feed conversion ratio but not with regard to body weight gain and ileal energy digestibility. A possible explanation could be the amount of metabolisable energy was slightly different between the diets, although this was not intended. Both diets were formulated to be isoenergetic and isonitrogenous assuming an AMEn value of 37.7 MJ/kg or 8843 kcal/kg for the rapeseed oil (Sauvant et al. 2002). However, the energetic value of the rapeseed oil has been reported to vary considerably (8000-8500 kcal/kg rapeseed oil) (Scheele et al. (1997), and the value used in our calculations may have overestimated the true amount of metabolisable energy. Another factor which could account in part for the better feed conversion of the diet with HRSF may be due to decreased ingredient segregation (higher gelatinisation) and therefore reduction of energy expenditure during prehension. The potential role of an *Eimeria* infection as a third factor that may have influenced the production performance results will be discussed in a separate paper (Granstad et al., manuscript in preparation).

The high starch digestibility in the diet with HRSF was due to inadvertent confounding factors, particularly the extent of starch gelatinisation, the use of isolated WS and possibly a reduced degree of lipid-amylose interactions. Because of this, our data cannot be used to reject our hypothesis that high ratio of starch to fat in the diet may impair digestibility and production performance. Further work is required to clarify this research question, taking into consideration the potentially confounding roles of feed processing and physical form of starch source. It seems however clear from our data that isolated starch is an excellent nutrient with regard to digestibility and production performance. The results also demonstrate a high capacity of the broiler chicken for digestion of diets independent of starch to fat ratio, even under unfavourable gastrointestinal tract environment.

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Table 1. *Experimental diets composition, calculated and analysed nutrient content (g/kg as fed)*

Ingredients	**HRSF***	**LRSF***
Wheat	412.6	412.6
Fish meal (72% CP)	100	100
Soybean meal (47.3% CP)	185	185
Wheat starch [1]	250	-
Rapeseed oil	-	87.4
Sand [2]	-	162.6
L-Lysine	2.8	2.8
DL-Methionine	2.8	2.8
L-Threonine	2	2
Limestone	12	12
Monocalcium phosphate	15	15
Sodium chloride	3	3
Titanium dioxide	5	5
Choline chloride	2	2
Mineral & Vitamin premix [3]	6.3	6.3
Enzyme (Rovabio) [4]	1.5	1.5
Calculated nutrient content		
Metabolisable energy (MJ/kg)	12.13	12.13
Dig. Lysine	12.9	12.9
Dig. Methionine	6.1	6.1
Dig Threonine	8.6	8.6
Analysed nutrient content		
Gross energy (MJ/kg)	16.20	15.95
DM (g/kg)	908	913
Starch (g/kg)	448	231
Fat (g/kg)	14.2	95.4
Crude Protein (g/kg)	211	211
Calcium (g/kg)	13.7	13.3
Phosphorous (g/kg)	8.1	7.9
Starch gelatinisation, g/kg starch	574.9	152.4
Starch: fat ratio	31.5: 1	2.4: 1

* **HRSF** and **LRSF:** high and low ratio of starch to fat

[1] Wheat starch, low gluten (AMILINA), ROQUETTE: Dry matter, 87%; Starch, 86%; Protein (Nx6.25), 0.35% max; Lipids, 0.1% max; Cellulose, 0.1% max and particle size distribution as follow: >200 µm, 2% max; >10 µm, 75% min.

[2] NC4AF, High Purity Quartz Sand, The Quartz Corp, Norway: SiO2, 99.99%; and particle size distribution as follow: >150 µm, <5%; 75-150 µm, 90%; <70 µm, 5%;

[3] Mineral and vitamin premix provided the following per kg diet: Fe, 53 mg; Mn, 125 mg; Zn, 83 mg; Cu, 15 mg; I, 0·75 mg; Se, 0·30 mg; retinyl acetate, 5.75 mg; cholecalciferol, 0.18 mg; dl-α-tocopheryl acetate, 80 mg; menadione, 10 mg; thiamine, 6 mg; riboflavin, 26 mg; niacin, 35 mg; calcium pantothenate, 26 mg; pyridoxine, 15 mg; cobalamin, 0.04 mg; biotin, 0.6 mg; folic acid, 5 mg.

[4] Enzyme Rovabio Excel Ap T-Flex, Adisseo, France provided the following per kg diet: Endo-1,4-β-xylanase: 33 000 visco units; Endo-1,3(4)-β-glucanase: 45 000 visco units; Endo-1,4-β-glucanase (cellulase) >9600 DNS units + 16 other enzyme activities obtained from a fermentation broth of *Penicillium funiculosum*.

Table 2. *Effect of varying ratios of starch: fat on the overall production performance of broilers*[1]

	10-15 days			15-24 days			24-28 days			10-28 days		
Diets	FI[3]	BWG[3]	FCR[3]	FI	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR
HRSF [2]	392	266	1.474	931	729	1.277	610	424	1.439	1893	1419	1.334
	± 12.0	± 8.3	± 0.04	± 15.2	± 8.3	± 0.01	± 4.9	± 5.8	± 0.02	± 28.5	± 20.1	± 0.00
LRSF [2]	411	272	1.511	948	660	1.436	651	433	1.503	1968	1400	1.406
	± 4.1	± 3.8	± 0.02	± 12.0	± 11.9	± 0.01	± 4.8	± 4.5	± 0.01	± 16.9	± 12.8	± 0.01
P-value*	0.1490	0.9770	0.1840	0.4880	**0.0330**	**<0.001**	**<0.001**	0.387	**0.0030**	**0.0210**	**0.1060**	**<0.001**

[1] Values are means ± SEM, (n= 12 replicate pens of 80 birds each)

[2] **HRSF** and **LRSF:** high and low ratio of starch to fat

[3] FI: Feed intake (g/bird); BWG: Body weight gain (g/bird); FCR: Feed conversion ratio: FI/BWG

* Means are considered significant at $P < 0.05$

Table 3. *Effect of varying ratios of starch: fat on amylase activity (Unit/g jejunal chyme), nutrient concentration in ileal digesta* [1] *and ileal digestibility of nutrients* [1] *and energy*

			Freeze-dried ileal digesta		Ileal digestibility coefficients		
Age	Diets	Amylase activity [3]	Starch (g/kg)	Fat (g/kg)	Starch	Fat	Energy [3]
16 days	**HRSF** [2]	75.9 ± 10.7	80.3 ±1.38	22.2 ± 0.12	0.950 ±0.01	0.575 ±0.03	-
	LRSF [2]	50.7 ± 10.6	58.1 ±1.33	56.1 ± 0.50	0.893 ±0.03	0.758 ±0.02	-
	P-value*	0.1112	**0.0665**	**< 0.001**	**0.0832**	**< 0.001**	-
29 days	**HRSF**	74.3 ± 11.1	42.3 ±0.46	18.0 ±0.10	0.978 ±0.00	0.690 ±0.01	0.766 ±0.01
	LRSF	51.1 ± 7.8	29.2 ±0.45	29.0 ±0.20	0.950 ±0.01	0.878 ±0.01	0.747 ±0.01
	P-value*	**0.0831**	**0.0148**	**< 0.001**	**0.0094**	**< 0.001**	0.1076

[1] Values are means ± SEM; n = 12 replicate pens with the average for 2 birds each

[2] **HRSF** and **LRSF**: high and low ratio of starch to fat

[3] n= 12 replicate pens of 1 bird each

* Means were considered significant at P < 0.05

Paper III

Interaction between starch source and degree of starch gelatinisation in broiler chickens: Effects on starch degradation rate and growth performance

K. Itani[a], J.Ø. Hansen[a], B. Kierończyk[b], A. Benzertiha[b], A.E. Kurk[a], P.P. Kurk[a], F. Sundby[a], L.T. Mydland[a], M. Øverland[a] & B. Svihus[a*]

[a] Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Aas, Norway; [b] Department of Animal Nutrition, Poznań University of Life Sciences, Poznań, Poland.

*Corresponding author:
Birger Svihus
birger.svihus@nmbu.no

ABSTRACT

1. A 2x2 factorial design was used to test the hypothesis that in pelleted diets, legume starch will be more digestion-resistant as compared to cereal starch, and that, increased gelatinisation through extrusion would reduce the difference in starch digestibility and growth performance between the two sources. Additionally, the study allowed for testing the hypothesis of the beneficial effect of a more gradual starch digestion or a low ratio of starch to nitrogen disappearance rate (**SNDR**) on broiler performance.
2. From 17 to 29 d, birds were randomly distributed among four dietary treatments consisting of either wheat (**W**) or faba bean starch fraction (**FBS**) as starch sources, and pelleting or extrusion as processing methods. Each treatment had 10 replicate pens with five birds per pen.
3. Extrusion cooking resulted in a more extensive starch gelatinisation compared to the pelleting process, as expected.
4. Birds fed W tended (*P <0.082*) to have better feed conversion ratio (**FCR**) than those fed FBS, while the difference between processing methods was insignificant. As a result, there was no interaction between starch source and processing method on FCR.
5. FBS in pelleted diet had lower starch digestibility and a slower starch disappearance rate compared to W in all intestinal segments (*P <0.05*). The interaction between starch source and processing method in all intestinal segments (*P <0.001*) demonstrated that FBS responded more to gelatinisation through extrusion than did the W. As a result, differences in starch digestibility between the W and FBS were reduced with extrusion.
6. Feeding slowly digestible starch did not improve feed conversion efficiency, nor did a lower ratio of SNDR.

Introduction

Because starch is the main energy source in broiler diets, impaired starch digestibility may adversely affect not only the cost of production, but also the health of the birds, the energy available for growth and the efficiency of feed conversion (Hetland et al. 2003, Engberg et al. 2004, Choct 2009). Grain legumes such as faba bean (*Vicia faba*) are considered a good source of nutrients and energy for poultry, however, its use in broiler diet has been limited to partial replacement due to its lower protein content compared to soybean meal (SBM) and to the presence of several anti-nutrients (Jezierny et al. 2010). In addition, because the type of crystal structure present in starch granules may influence its digestibility (Zhang et al. 2006, Ao et al. 2007), *in vitro* studies (Weurding 2002, Hoover and Zhou 2003, Li et al. 2018) showed that type C starch from legumes is more slowly and to a lesser extent digested than type A starch from cereals (Sun et al. 2006). Similarly, studies with broiler chickens have shown that starch from legumes is generally more resistant to intestinal degradation than cereal starch (Carre et al. 1991, Carré et al. 1998, Wiseman 2006). The significant progress in plant breeding, combined with use of numerous processing techniques (dehulling, pelleting and extrusion), were shown to have great potential in enhancing the nutritional and energetic value of faba beans, consequently improving broiler performance(Marquardt and Campbell 1973, Lacassagne et al. 1988, Diaz et al. 2006, Crépon et al. 2010, Hejdysz et al. 2016a). Air classification is another processing technique for the dry separation of particles of different densities and shapes, for example from finely ground dehulled faba bean, into a protein concentrate (FBP; light fraction) and a starchy flour (FBS; dense fraction) (Vose et al. 1976). These fractions can thus be used as a concentrated energy source or a protein supplement in broiler diets.

While high starch digestibility is always desirable, it has been proposed that feeding gradually or slowly digestible starch may improve the efficiency of feed conversion in broilers (Weurding 2002, Del Alamo et al. 2009, Liu and Selle 2015). These researchers hypothesised that rapidly digested starch (defined as the starch that is almost completely digested by the time it reaches the distal jejunum) would not provide enough energy in the form of glucose to the enterocytes in the lower part of the small intestine compared to slowly digested starch. Consequently, a larger proportion of amino acids will be used as an energy source for the enterocytes instead of for muscle growth. Contrary, due to its longer supply of glucose, gradually digested starch (digested at the distal ileum) may spare amino acid oxidation, and thus result in improved growth performance of the bird (Weurding et al. 2003a). Results confirming the aforementioned hypothesis however, were not always consistent. For instance, Weurding et al. (2003b) reported that feeding pea-corn based diets (slowly digestible starch of a digestion rate of 1.05 h^{-1}) for broilers resulted in a 1.9% improvement in FCR compared to feeding tapioca-corn diets (rapidly digestible starch of a digestion rate of 1.99 h^{-1}). On the other hand, Del Alamo et al. (2009) reported that feeding young broilers a diet with a starch digestion rate of 1.8 h^{-1} impaired performance (FCR = 1.668), but that of 2.17 h^{-1} and even 2.56 h^{-1} improved growth rate.

Manipulating the rate of starch digestion may be achieved by using two sources of starch differing in their susceptibility to amylase hydrolysis, i.e. different digestion rate, which can be determined *in vitro*, in conditions simulating the digestive tract of broiler chickens (Weurding et al. 2003b). Additionally, the same starch source can be subjected to pelleting or extrusion, thereby altering starch properties differently (small extent or almost complete gelatinisation, respectively). This may increase starch digestibility, as has been shown earlier (Zimonja and Svihus 2009), and recently in extruded compared to pelleted wheat-based diets (Itani and Svihus 2019).

The hypothesis tested was that in pelleted diets, faba bean starch (**FBS**) will be more digestion-resistant as compared to wheat (**W**) and that the increased gelatinisation through extrusion may change the availability of starch, thereby reducing the difference in starch digestibility and performance between the two starch sources. In addition, the hypothesis of the beneficial effects of a more gradual starch digestion on broiler performance was tested.

Materials and methods

According to the Polish law and the EU directive (no 2010/63/EU) the experiments conducted within the study do not require approval of the Local Ethical Committee for Experiments on Animals in Poznań.

Processing of main ingredients and experimental diets

The faba beans were first cracked using a roller mill (DT900-12; CPM-Roskamp, Waterloo, IA, United States) with 8 mm gap between the rolls and cleaned from dust using a pre-cleaner Damas Vibam type 1013 (Damas A/S, Faaborg, Denmark). Next, the dehulled beans (cotyledons) were milled with a Contraplex 630 C pin mill (Hosokawa Alpine, Augsburg, Germany) and finally, the flour was air classified using an Air Classifier 500 ATP (Hosokawa Alpine, Augsburg, Germany) to produce a light protein-rich fraction and a heavy starch-rich fraction (**Table 1**). The wheat was pin-milled as described above, without further processing. The particle size of the W and the FBS is presented in **Figure 1.** The SBM was ground to pass through a 1-mm sieve in a hammer mill (Münch-Edelstahl, Wuppertal, Germany licensed by Bliss, USA, 18.5kW, 3000 RPM) before being mixed with other ingredients. Experimental diets were processed at the Centre for Feed Technology (Fôrtek), Norwegian University of Life Sciences, Ås, Norway, and were formulated to be isonitrogenous and isoenergetic and to meet or exceed Ross 308 strain recommendations (Aviagen 2014) for major nutrients (**Table 2** and **Table 3**). The diets contained Titanium dioxide, (**TiO_2**) as a digestibility marker and cellulose powder was used to balance the diets for fibre content. The mash was steam-conditioned in a double pass pellet-press conditioner (Münch-Edelstahl, Wuppertal, Germany) at 81°C and then pelleted using a pellet press (Münch-Edelstahl, Wuppertal, Germany, 1.2 t/h, 2×17 kW, RMP 350) equipped with a 3 mm die (42 mm thickness), at a production rate of 400 and 200 kg/h for the W- and FBS- based diet respectively. Specific energy consumption values (kWh/t) were 38 and 77 for the W- and FBS-based diets, respectively. Post-pelleting temperatures were 89 and 94°C for the W- and FBS-based diet, respectively and were measured by collecting a sample of hot pellets from immediately below the pellet press into an insulated box fitted with a thermometer. The extruded diet was steam heated at 89°C in an extruder pre-conditioner (Bühler BCTC 10, Uzwil, Switzerland) prior to processing in a co-rotating twin-screw extruder (Bühler BCTG 62/20 D, 5 sections, 72 kW DC, Uzwil, Switzerland) fitted with 12 dies x 3 mm and with a feeder rate of 145 kg/h for the W- and FBS-based diet, respectively. The temperatures in the five sections of the extruder were 92, 112, 95, 90, and 64°C for the W diet and 95, 110, 100, 96, and 64°C for the FBS diet. Specific mechanical energy values (KWh/t) were 65 and 62, and die temperatures were 91 and 95°C for the W- and FBS-based diets, respectively. Moisture content during extrusion was kept at around 290 g/kg by addition of steam and water (ambient temperature) in amounts of 60 g/kg and 100 g/kg in the conditioner. During pelleting, around 43 g/kg of steam were added in the conditioner to achieve an average total moisture of 150 g/kg.

Birds, housing and management

A total of 400 one-day-old male broilers (Ross 308) were randomly allocated to 40 floor pens (1 x 1 m) that were bedded with chopped wheat straw (7-15 cm length) and contained 10 birds each. The pens were arranged in the centre of an environmentally-controlled broiler house (PIAST PASZE Sp. z o.o., Experimental Unit no. 0616, Olszowa, Poland) that contained 9000 birds of the same age as those in the experiment. A temperature of 33°C was maintained during the first week, then reduced by 3-4°C weekly to a minimum temperature of 21°C. The birds were maintained on a commercial pelleted diet produced by Piast Pasze feed mill (Lewkowiec, Poland) until 16 d, and fresh water was provided ad libitum throughout the experimental period. At 17 d, the birds were randomly distributed among 4 dietary treatments using 10 replicate pens per treatment and 5 birds per pen (after reducing the number of birds from 10 to 5). Treatments consisted of a control and an experimental diet with W or FBS as the main dietary starch source, respectively. These diets were either steam-pelleted or extruded, thus constituting a 2 x 2 factorial experiment.

Performance measurement

The birds and the feed were weighed on a pen basis on d 17 and 29. Performance data was adjusted for mortality.

Sample collection

At 30 d, 20 birds (2 birds/replicate pen) per treatment were weighed, killed by cervical dislocation and the gizzard removed, freed from surrounding fat and weighed full and empty. Next, using clamping forceps, the jejunum and ileum were clamped at three points (proximal, mid and distal part) to prevent the passage of contents along the intestine, then weighed. The jejunum was defined as the segment from the end of the duodenal loop to Meckel's diverticulum, and the ileum as the section from Meckel's diverticulum to the ileo-cecal junction. Each of the two segments was then divided into two parts of equal length: upper and lower jejunum (Uj and Lj), upper and lower ileum (Ui and Li) and the contents of each part were expressed by gentle manipulation into a pre-weighed plastic container and stored at −20°C until analysis. To measure enzyme activity, around 200 mg of fresh digesta from the Lj were transferred to a 2 mL Sarstedt tube, frozen on dry ice then stored at −80°C until analysis.

Chemical analyses

Representative feed samples (n=3) were ground on a cutting mill (Pulverisette 19, Fritsch Industriestr. 8, 55743 Idar-Oberstein, Germany) through a 0.5 mm sieve. Gross energy (GE) was determined using an adiabatic bomb calorimeter (Parr 6400, Moline, USA) standardized with benzoic acid. Dry matter and ash content of the feed were determined after drying overnight at 105°C and after 6 h ashing at 550°C, respectively. Nitrogen content was determined by the Dumas method using a Vario El Cube (Elementar Analysensysteme GmbH, Hanau, Germany 2016). Amino acids in the diets were analysed on a Biochrom 30 amino acid analyser (Biochrom Ltd., Cambridge, UK). Ether extract was determined after extraction with 80% petroleum ether and 20% acetone in an Accelerated Solvent Extractor from Dionex (ASE200; Sunnyvale, CA, USA). Fibre content was determined using a fibre analyser system (Ankom200; ANKOM Technologies, Fairport, NY, USA) with filter bags (Ankom F58; ANKOM Technologies). Starch content was analysed enzymatically based on the use of thermostable α-amylase and amylo-glucosidase (McCleary et al. 1994) and TiO_2 content was determined as described by Short et al. (1996). Freeze-dried jejunal and ileal contents were pulverized using a mortar and pestle, and the contents from two birds per replicate pen were pooled and analysed in duplicates for starch (without 80 % ethanol washing), nitrogen and TiO_2 as described above. Intestinal samples from the lower jejunum were taken from one bird per replicate pen and were prepared as described by Pérez de Nanclares et al. (2017) for enzyme activities analysis. Amylase and trypsin activities were assayed colorimetrically using amylase and trypsin commercial assay kits (Abcam, Cambridge, UK) according to manufacturer's instructions. Activities of amylase and trypsin were expressed as unit/g jejunal chyme. The particle size distribution of the W and FBS was determined by the laser diffraction method using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) as described by Hetland et al. (2002). The degree of starch gelatinisation (DG; as a proportion of total starch) was measured by differential scanning calorimetry (DSC 823e Module, Mettler-Toledo, Switzerland) as described by Kraugerud and Svihus (2011).

Calculations

The apparent digestibility coefficients of starch and nitrogen were calculated using the following formula:

$$\text{Apparent digestibility coefficient} = \frac{\left(\frac{\text{NT}}{\text{Ti}}\right)\text{diet} - \left(\frac{\text{NT}}{\text{Ti}}\right)\text{digesta}}{\left(\frac{\text{NT}}{\text{Ti}}\right)\text{diet}}$$

$\left(\frac{\text{NT}}{\text{Ti}}\right)$ is the ratio of the nutrient to TiO_2 in the diet or in the digesta.

Apparent disappearance rates of starch and nitrogen along the intestinal tract were calculated using the following formula (Sydenham et al. 2017):

Apparent disappearance rate (g/bird/day) = dietary concentration of the nutrient (g/kg) x feed intake over the final 24 h of feeding (g/bird) x digestibility coefficient of the nutrient

Starch: nitrogen disappearance rate ratios in the small intestine were calculated from this data.

Statistical analysis

Statistical analyses were carried out using the statistical software R version 2.3.2. A two-way analysis of variance (ANOVA) was performed to determine the main effects and interactions of starch sources and processing methods (as independent variables) on growth parameters, digestive characteristics, nutrient digestibility and enzyme activities. Means were separated by Tukey post-hoc test and differences were considered significant at $P < 0.05$. Pen means (5 birds) were used as the experimental unit for performance data.

Results

Ingredients and diets:

The chemical composition (**Table 1**) of the FBS and the FBP showed that air classification (following pin milling) is an efficient technique to produce starch- or protein-rich fractions that can be used as alternatives to conventional feedstuffs. While FBS had higher starch and protein content then the W, its fibre fraction was very low compared to that of W. For this reason, cellulose powder was used to balance the diets for fibre content. In addition, due to the difference in amino acid profile of the W and FBS (data not shown), the diets (**Table 3**) were balanced accordingly using synthetic amino acids.

Dissection: Gizzard and small intestine characteristics:

Gizzard empty weight was greater (*P =0.035*) for birds fed the FBS-diet, while no difference (*P >0.05*) in the relative weight of the jejunum and ileum (with contents) was observed between treatment groups (**Table 4**).

Growth performance:

Mortality was very low (less than 2%) and not related to dietary treatments. Growth performance results are shown in **Table 5**. BWG was not affected (*P >0.05*) by starch source, although birds fed the FBS diet had significantly higher (*P =0.007*) feed intake than those fed the W diet. As a result, the FBS group tended to (*P =0.082*) be less efficient in feed conversion compared to the W group. Birds fed extruded diets had higher weight gain (*P =0.032*) compared to those fed pelleted diets, partly due to a simultaneous increase in feed intake (*P =0.001*). As a result, FCR was similar (*P =0.209*) for extruded and pelleted diets. Overall, there was no interaction between starch source and feed processing on FCR.

Apparent starch digestibility and starch disappearance rate (SDR) along the small intestine:

In all intestinal segments, starch digestibility was only significantly lower for the FBS compared to the W in pelleted diets, and the difference was more pronounced in the anterior part of the jejunum (**Table 6**). This resulted in a significant (*P <0.05*) interaction between starch source and processing method in the Uj, Ui and Li, and in a tendency for an interaction (*P =0.057*) in the Lj. As shown in **Table 8**, along all intestinal segments, FBS had significantly slower SDR compared to the W only in pelleted diets. Extrusion cooking on the other hand, increased SDR in FBS compared to W diet resulting in a significant (*P <0.001*) interaction effect between starch source and processing method.

Apparent nitrogen digestibility and nitrogen disappearance rate (NDR) along the small intestine:

Compared to extrusion processing, pelleting significantly improved (*P =0.012*) nitrogen digestibility in the Uj, while neither starch source nor processing method (*P >0.1*) affected nitrogen digestibility in the Lj or Ui (**Table 7**). However, nitrogen digestibility in the Li was significantly higher (*P =0.027*) in birds fed the FBS-based diet compared with those fed the W-based diet. Compared to extrusion, pelleting increased (*P =0.019*) NDR in the upper jejunum, while no major differences were noted between the treatments distal to this segment (**Table 9**).

Ratio of starch: nitrogen disappearance rate (SNDR) along the small intestine:

In both jejunal sections, extrusion cooking significantly (*P <0.001*) widened the ratio of SNDR compared to pelleting (**Table 10**). In the upper and lower ileum, significant (*P <0.001*) interactions were observed, where a lower ratio of SNDR was noted in birds given FBS only when the diets were pelleted.

Enzyme activities:

As shown in **Table 11**, there was a tendency for higher (*P >0.055*) amylase activity in jejunal digesta of birds fed FBS compared to those fed the W diet. On the other hand, feeding W diet increased (*P =0.003*) trypsin activity compared to feeding FBS diet. Trypsin activity was also influenced by feed processing. Thus, birds fed extruded diet had significantly higher (*P <0.019*) trypsin activity than those fed pelleted diets.

Discussion

As expected, and in agreement with earlier (Moritz et al. 2005, Zimonja and Svihus 2009) and recent observations (Itani and Svihus 2019), extrusion technology resulted in a more extensive starch gelatinisation (on average: 83% vs 20%) compared to the pelleting process. Conventional pelleting is run at a low total water content and moderate temperature; thus, it will only have a limited contribution to starch gelatinisation (Svihus et al. 2005). The higher water content and temperature during the extrusion processing, cause irreversible and more severe disruption of intra- and inter-molecular hydrogen bonds, resulting in a loss of crystallinity and amylose leaching out of the granule (Lund and Lorenz 1984, Hoover 1995).

While pelleting resulted in a similar amount of starch gelatinisation in the W and FBS, whereas extrusion processing increased the level of gelatinised starch as a proportion of total starch in the FBS compared to the W diet. The reason for this is unclear, but may partly be explained by the unintended difference in particle size distribution between the two sources (Marshall 1992, Al-Rabadi et al. 2011). Hasjim et al. (2013) reported that rice flour samples with larger particle size have greater physical barrier to heat and water diffusion than smaller particles, which may be the cause for the higher gelatinisation degree in favour for the FBS diet.

Starch digestibility coefficients and starch disappearance rates along the intestinal tract confirmed that, with limited gelatinisation, legume starch is more resistant to intestinal degradation than cereal starch. Even though the difference in the digestion profile was more pronounced in the upper part of the small intestine, starch from both sources was digested to a large extent in the lower ileum. In contrast, extrusion cooking improved the digestibility of legume-starch as a result of the destruction of the crystalline structure in the granules (Hejdysz et al. 2016a, Hejdysz et al. 2016b), and increased its disappearance rate in all intestinal segments. Unexpectedly, however, and contrary to what has been reported recently (Itani and Svihus 2019), there was no difference in starch digestibility between the pelleted and extruded W diet. This suggest the presence of other factors that may have increased the digestion of W starch and masked the effect of increased gelatinisation on starch availability, consequently leaving no room for improvement.

Although the hammer mill is the most common method for grinding feed ingredients, in the current experiment and due to necessity, the W was ground using a pin mill. Therefore, by applying the same milling conditions to the wheat and to the dehulled faba bean, the confounding effect of different grinding methods was avoided. Because very fine grinding also increases the efficiency of fractionation (Sosulski et al. 1988), the pin mill is generally used prior to air classification due to its capacity to produce the required fine particles (Vasanthan and Bhatty 1995, Létang et al. 2002, Wu and Nichols 2005). It is worth mentioning that the particle size distribution of the pin-milled W in the current experiment was comparable to that of digesta particle size in the duodenum of a group of broilers exhibiting high starch digestibility (Hetland et al. 2002).

Moreover, studies have shown that a well-developed gizzard can efficiently grind coarse wheat particles to very fine particle sizes, thereby enhancing nutrient digestion (Svihus 2006, Amerah et al. 2007) and feed utilisation. Because the diets in the current experiment did not stimulate gizzard development, it is reasonable to suggest that the degree of fineness of the W (caused by the pin mill) has outweighed the need for a well-functioning gizzard to grind the feed to facilitate digestion. As a result, starch digestibility in the W was almost complete, even in pelleted diets.

The resistance of legume starch to digestion compared to cereal starch is highlighted by the difference in particle size distribution between the two starch sources. As shown by the particle size analysis (**Figure 1**), and due to the effect of air classification, the FBS was finer than the W, with volume weighted mean of 50 and 240 μm and surface weighted mean of 21 and 26 μm, respectively. This characteristic, as mentioned earlier, is generally known to increase the susceptibility of starch to enzymatic hydrolysis, i.e. the rate of starch digestion (Angelidis et al. 2016). However, even with a reasonably high ileal starch digestibility for the FBS in the pelleted diet, W was still more digestible. This explains the significant interaction between starch source and processing method on starch digestibility throughout the intestinal segments.

It is known that the ratio of amylose to amylopectin is higher in legume compared to cereal grains (Bhatty 1974, Grant et al. 2002, Ambigaipalan et al. 2011), and that high amylose content is associated with reduced starch digestibility *in vitro* and *in vivo* (Topping et al. 1997, Zhou and Kaplan 1997, Ankrah et al. 1999, Regmi et al. 2011). Even high-amylose cereal starch, for example from hull-less barley, has been shown to be hydrolysed at a significantly lower rate compared to waxy genotype (Li et al. 2004). Compared to the more branched amylopectin molecule, amylose has a lower molecular weight, a more compact and linear structure and thus, a lower surface area for amylase (Thorne et al. 1983). Naivikul and D'appolonia (1979) found that, compared to cereal starch, legume starch exhibited higher pasting temperature and viscosity, indicating higher resistance to swelling and rupture.

It was observed that amylase activity was higher in birds fed the FBS diet, which may be a reflection of the higher amount of starch in freeze-dried intestinal contents (**Figure 2**), as reported before (Karasov and Hume 1997, Engberg et al. 2004). The higher amylase activity in the FBS diet did not result in an increase in starch digestibility relative to the W diet, suggesting inadequate amylase secretion. Alternatively, amylase may not be a limiting factor *per se*, but it is the amylase-resistant nature of legume starch when minimally gelatinised. This postulation is supported by the results of Yutste et al. (1991) and Weurding et al. (2001) who, using mash diets, reported lower ileal starch digestibility in semi-purified bean starch and horse bean, respectively compared to wheat starch in broiler chickens. The significantly higher starch digestibility in the extruded compared to the pelleted FBS diet also corroborates the above suggestion especially that amylase activity did not differ between the two treatments.

Because both diets contained similar amount of SBM, it is reasonable to postulate that the difference in nitrogen digestibility may be attributed to the protein fraction in the starch sources. Accordingly, ileal apparent nitrogen digestibility was higher in the FBS diet compared to the W diet, indicating that bean protein (possibly due to the finer particle size of FBS) was more accessible to digestion compared to that of W. Corroborating this, Crévieu et al. (1997) reported that, feeding broilers finely milled pea seeds significantly improved the apparent ileal protein digestibility (89.5 vs 70.2 %) compared to coarse milling, probably due to the larger surface area of fine particles to digestive enzymes. On the other hand, fine grinding of wheat did not improve ileal protein digestibility compared to coarse grinding in a wheat-SBM based diet fed to young broilers (Péron et al. 2005). Faba bean protein has also been reported to be equally digestible as SBM protein or soy protein concentrate (Gunawardena et al. 2010, O'Neill et al. 2012). Moreover, dehulling, low-tannin content, and heat treatment were also described as contributors to the significant increase in protein digestibility of legumes, particularly faba bean and pea seeds (Carré et al. 1987, Alonso et al. 2000, Crépon et al. 2010). The lower trypsin activity in the FBS fed birds may explain the lower need for excess enzymes when the digestibility of the substrate is high (Murugesan et al. 2014).

In the present experiment, feeding FBS, a source of slowly or more gradually digestible starch compared to W, seems not to improve feed conversion efficiency. In fact, there was a tendency (*P =0.082*) for FBS to impair FCR compared to W. This is not in line with previous and recent suggestions (Weurding 2002, Liu and Selle 2015). It should also be mentioned that, although pelleting resulted in a numerically lower FCR compared to extrusion, the difference was not statistically significant (*P* = 0.209). According to the hypothesis of negative effect of rapidly digested starch on feed efficiency, extrusion should have resulted in significantly poorer FCR compared to pelleting, but as stated above, this was not the case. Li et al. (2008) investigated the effects of different starch sources on the appearance of amino acids and glucose in the portal circulation of pigs. They found that slowly digestible starch (resistant starch) significantly reduced glucose and amino acids net absorption into the portal vein. Accordingly, it was suggested that resistant starch may increase the catabolism of amino acids by the small intestine, which as a result will reduce the efficiency of nutrient utilisation and impair pig performance. Moreover, Hejdysz et al. (2017) found that offering pea in extruded form (up to 500 g/kg diet) improved broiler performance, nutrient and energy utilisation and FCR compared to raw form. Also, although apparent metabolisable energy (AME) was not measured in the current study. Truong et al. (2016) reported that slowly digestible starch may improve AME and nitrogen corrected AME (AMEn), however, more recent experiment from the same lab showed significant improvement in AME, ME:GE ratio, N retention and AMEn with 45% inclusion of rapidly digested purified maize-starch in a maize-SBM based control diet (Moss et al. 2018).

According to Liu and Selle (2015), the starch digestion dynamics should be treated in combination with that of protein, because of the intricate relationship between these macronutrients and their effect on growth efficiency. As stated by Sydenham et al. (2017), calculations of SNDR ratio is one approach to quantify starch and nitrogen digestive dynamics. In several studies conducted at the same institution, different conclusions were derived regarding the relation of starch: nitrogen disappearance rate and broiler performance. As stated by Sydenham et al. (2017), some studies found that broiler performance improved linearly with a lower ratio of SNDR, while the same article, Sydenham et al. (2017), concluded that this relationship is quadratic, and emphasized the importance of an optimal balance between the digestive dynamics of the two components in the proximal jejunum. Truong et al. (2017) on the other hand, did not detect any significant difference in any of the performance parameters between broilers fed six varieties of sorghum exhibiting different ratios of SNDR in all intestinal segments. In the current experiment, pelleting had a narrower ratio of SNDR compared to extrusion, particularly in the proximal and distal jejunum, however, no significant difference in FCR was detected. Gilbert et al. (2007) concluded based on the expression levels of nutrient transporters that, the jejunum is the primary site of sugar assimilation in the chicken intestine, while the ileum is a more important site for amino acid assimilation. Thus, a more rapid starch digestion (i.e. higher ratio of SNDR) would be logical to meet the higher energy demands of the jejunum. This may simultaneously spare more amino acids from oxidation, thus increasing their appearance in the portal circulation, as seen recently by Yin et al. (2019). Consequently, a smaller portion of the amino acids will be used as fuel for the enterocytes in the ileum, a relatively less demanding tissue in terms of digestion and absorption compared to the jejunum (Gao et al. 2017). Clearly, the findings are inconsistent and sometimes contradictory due to the complexity of the hypothesis and to the presence of confounding factors. Further well-designed experiments are needed to clarify and to understand the relationship between the digestive dynamics of starch/protein and its effect on broiler performance.

In conclusion, FBS in pelleted diet had a lower starch digestibility and a slower starch disappearance rate compared to W in all intestinal segment. The magnitude was more pronounced in the upper jejunum. The interaction between starch source and processing method in all intestinal segments demonstrated that legume starch responded more to gelatinisation through extrusion than did the cereal starch. As a result, differences in starch digestibility between the W and FBS were reduced with extrusion. Feeding slowly digestible starch did not improve feed conversion efficiency, nor did a lower ratio of SNDR.

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Table 1. *Analysed chemical composition (g/kg) of the wheat (W), dehulled faba bean parent meal (FBPM), and the air-classified faba bean starch (FBS) and protein (FBP) fractions*

Item	W	FBPM	FBS	FBP
Dry matter	895	860	902	925
Crude protein	122	276	159	585
Starch	597	309	672	81
Ether extract	12.2	17.5	7.2	31
NDF	95	48.6	19.6	91

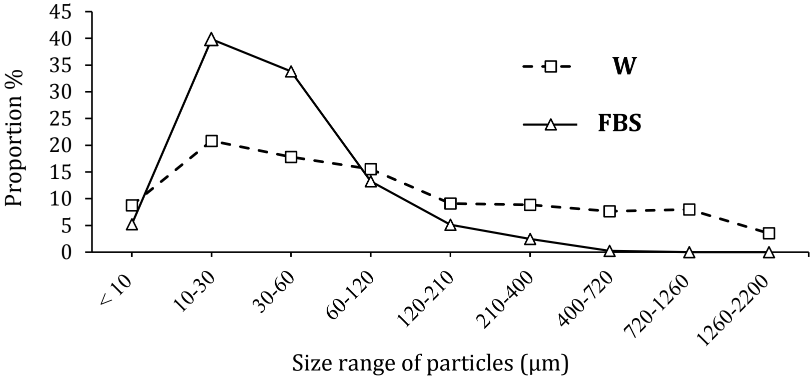

Figure 1. Particle-size distribution of the main starchy ingredients. W, wheat and FBS, faba bean starch (air-classified fraction)

Table 2. *Experimental diets composition, analysed and calculated nutrient content*

Ingredients, g/kg (as fed)	Cereal	Legume
Wheat (W)	582	--
Faba bean starch (FBS)	--	512
Soybean meal [1]	274	275.6
Cellulose powder [2]	--	70
Rapeseed oil	75	76
Limestone	14.77	15.04
Monocalcium phosphate	16.79	22.28
L-Lysine	8	1
DL-Methionine	6.09	5.61
L-Threonine	4	3.6
Sodium chloride	4.76	4.29
Titanium dioxide	5	5
Choline chloride	1.96	1.95
Mineral & Vitamin premix [3]	6.13	6.13
Enzyme (Rovabio) [4]	1.5	1.5
Analysis	Pelleted - Extruded	Pelleted - Extruded
Dry matter	904 - 934	906 - 923
Starch gelatinisation [5]	209 - 715	207 - 943
Gross energy (MJ/kg DM)	19.7	19.6
Starch (g/kg DM)	370	374
Crude Protein (g/kg DM)	239	237
Fat (g/kg DM)	90	90
NDF (g/kg DM)	110	118
Lysine (g/kg DM)	16	15
Methionine (g/kg DM)	7.8	7.8
Threonine (g/kg DM)	9.6	10.3
Calculated nutrient content		
Metabolisable energy	12.6	12.7
Calcium (g/kg)	9.7	10.5
Available Phosphorous	5.0	5.4

[1] Ground to pass a 1-mm screen

[2] SANACEL® 150, CFF GmbH & Co. KG, Gehren. Germany.

[3] Mineral and vitamin premix provided the following per kg diet: Fe, 50 mg; Mn, 122 mg; Zn, 80 mg; Cu, 14 mg; I, 0·72 mg; Se, 0·28 mg, retinyl acetate, 5.72 mg; cholecalciferol, 0.15 mg; dl-α-tocopheryl acetate, 78 mg; menadione, 8 mg; thiamine, 5 mg; riboflavin, 24 mg; niacin, 32 mg; calcium pantothenate, 24 mg; pyridoxine, 13 mg; cobalamin, 0.03 mg; biotin, 0.5 mg; folic acid, 4 mg.

[4] Enzyme Rovabio Excel Ap T-Flex, Adisseo, France provided the following per kg diet: Endo-1,4-β-xylanase: 33 000 visco units; Endo-1,3(4)-β-glucanase: 45 000 visco units; Endo-1,4-β-glucanase (cellulase) >9600 DNS units + 16 other enzyme activities obtained from a fermentation broth of Penicillium funiculosum.

[5] Starch gelatinisation: g/kg of total starch

Table 3. *Analysed amino acid composition (g/kg DM) of the diets*

	Cereal			Legume		
Essential amino acids	Pelleted	-	Extruded	Pelleted	-	Extruded
Arginine	12.6	-	12.3	15.5	-	14.6
Histidine	4.6	-	4.5	5.1	-	4.9
Isoleucine	7.7	-	7.4	8.4	-	7.8
Leucine	13.8	-	13.5	15.0	-	14.0
Lysine	15.8	-	16.8	15.6	-	14.5
Methionine	7.3	-	8.3	8.7	-	7.0
Phenylalanine	9.2	-	9.2	9.6	-	9.1
Threonine	9.2	-	10.0	10.6	-	9.9
Valine	8.4	-	8.3	9.3	-	8.7
Non-essential amino acids						
Alanine	6.5	-	6.3	7.3	-	6.9
Aspartic acid	18.9	-	17.7	22.9	-	20.7
Cystein	2.6	-	2.6	2.6	-	2.4
Glutamic acid	40.7	-	42.0	38.0	-	37.0
Glycine	6.7	-	6.6	7.4	-	6.9
Proline	12.1	-	12.5	10.4	-	9.9
Serine	8.9	-	8.7	9.5	-	9.0
Tyrosine	4.4	-	4.7	5.2	-	5.2
Total amino acid	189.2	-	191.6	201.1	-	188.5

Table 4. *The effect of starch source and processing method on body weight, gizzard characteristics and relative weight of jejunum and ileum with content of 30-d-old broilers*[1]

Starch source	**Processing**	Body weight	Gizzard		Jej+ile relative weight[3]
			Empty weight	Relative weight[2]	
W	Pelleting	2331	17.3	7.4	41.1
FBS	Pelleting	2328	19.0	8.2	42.3
W	Extrusion	2376	17.3	7.3	42.8
FBS	Extrusion	2369	19.0	8.0	40.9
	√MSE[*]	154.21	2.41	0.94	5.24
Starch source					
W		2353	17.3	7.4	41.9
FBS		2349	19.0	8.1	41.6
Processing					
Pelleting		2330	18.2	7.8	41.7
Extrusion		2372	18.2	7.7	41.9
P-value					
Starch source		0.921	**0.035**	**0.074**	0.840
Processing		0.388	0.987	0.333	0.922
Diet x Processing		0.974	0.989	0.613	0.346

[1] Values are means of 10 replicate cages of 2 birds each

[2] Relative empty weight: expressed as g/kg body weight

[3] Relative full weight of the jejunum and ileum: expressed as g/kg body weight.

[*] √MSE: square root of means square error in the analysis of variance.

Table 5. *The effect of starch source and processing method on the growth performance[1] of male broilers from 17 to 29 d*

Starch source	**Processing**	Body weight gain	Feed intake[2]	Feed per gain
W	Pelleting	1514	1681	1.124
FBS	Pelleting	1509	1772	1.175
W	Extrusion	1562	1812	1.164
FBS	Extrusion	1601	1908	1.192
	√MSE[*]	99.61	103.22	0.07
Starch source				
W		1538	1747	1.144
FBS		1555	1840	1.184
Processing				
Pelleting		1512	1727	1.150
Extrusion		1582	1860	1.178
P-value				
Starch source		0.587	**0.007**	**0.082**
Processing		**0.032**	**0.001**	0.209
Diet x Processing		0.492	0.946	0.539

[1] Values are means of 10 replicate cages of 5 birds each

[2] On a dry matter basis

[*] √MSE: square root of means square error in the analysis of variance.

Table 6. *The effect of starch source and processing method on starch digestion along the intestinal tract of 30-d-old male broilers*[1]

			Jejunum		**Ileum**	
Starch source	**Processing**		Upper	Lower	Upper	Lower
W	Pelleting		0.921 **a**	0.947	0.981 **a**	0.998 **a**
FBS	Pelleting		0.826 **b**	0.912	0.940 **b**	0.972 **c**
W	Extrusion		0.879 **ab**	0.973	0.994 **a**	0.994 **ab**
FBS	Extrusion		0.902 **a**	0.971	0.985 **a**	0.987 **b**
		√MSE*	0.051	0.027	0.020	0.006
Starch source						
W			0.900	0.960	0.988	0.996
FBS			0.864	0.942	0.962	0.980
Processing						
Pelleting			0.873	0.930	0.959	0.985
Extrusion			0.891	0.972	0.989	0.991
P-value						
Starch source			0.032	0.038	0.001	<0.001
Processing			0.299	<0.001	<0.001	0.009
Diet x Processing			**0.001**	**0.057**	**0.018**	**<0.001**

[1] Values are means of 10 replicate cages of 2 birds each.

*√MSE: square root of means square error in the analysis of variance.

[a, b, c] Means within column followed by different letters are significantly different ($P < 0.05$).

Table 7. *The effect of starch source and processing method on nitrogen digestion along the intestinal tract of 30-d-old male broilers*[1]

		Jejunum		**Ileum**	
Starch source	**Processing**	Upper	Lower	Upper	Lower
W	Pelleting	0.370	0.582	0.711	0.813
FBS	Pelleting	0.305	0.552	0.735	0.832
W	Extrusion	0.255	0.538	0.737	0.823
FBS	Extrusion	0.254	0.588	0.733	0.848
	$\sqrt{}$MSE[*]	0.096	0.068	0.046	0.030
Starch source					
W		0.309	0.560	0.725	0.818
FBS		0.279	0.570	0.734	0.840
Processing					
Pelleting		0.338	0.567	0.724	0.822
Extrusion		0.255	0.563	0.735	0.836
P-value					
Starch source		0.341	0.651	0.537	**0.027**
Processing		**0.012**	0.853	0.437	0.170
Diet x Processing		0.298	**0.074**	0.346	0.774

[1] Values are means of 10 replicate cages of 2 birds each.

[*] $\sqrt{}$MSE: square root of means square error in the analysis of variance.

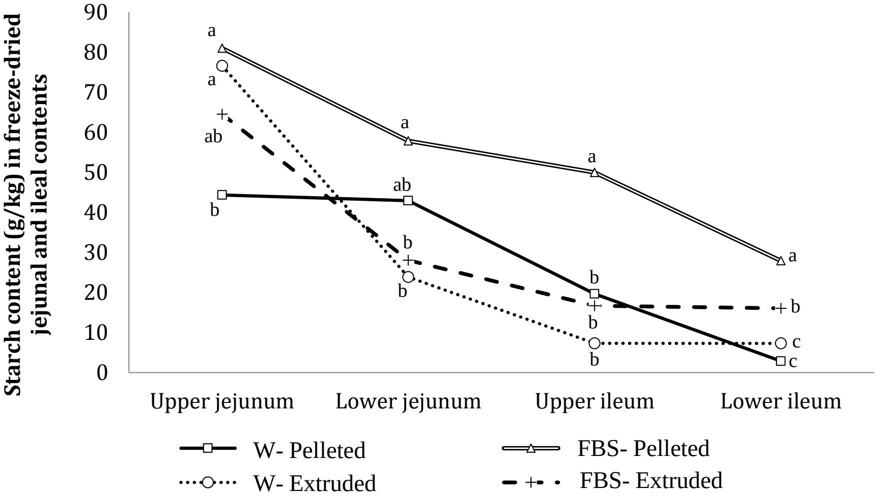

Figure 2. Means (n=10) of starch content (g/kg) in freeze-dried intestinal contents. Means with different letters within intestinal sections are significantly different (*P* < 0.05)

Table 8. *The effect of starch source and processing method on starch disappearance rate (g/bird/day) along the intestinal tract of 30-d-old male broilers*[1]

		Jejunum		**Ileum**	
Starch source	**Processing**	Upper	Lower	Upper	Lower
W	Pelleting	66.6 **b**	70.0 **c**	71.4 **c**	72.4 **c**
FBS	Pelleting	56.4 **c**	62.2 **d**	64.1 **d**	66.3 **d**
W	Extrusion	72.9 **b**	80.7 **b**	82.5 **b**	82.5 **b**
FBS	Extrusion	79.8 **a**	85.9 **a**	87.1 **a**	87.3 **a**
	√MSE[*]	5.17	3.44	3.58	3.28
Starch source					
W		70.0	75.1	77.5	77.7
FBS		68.1	74.1	75.6	76.8
Processing					
Pelleting		61.2	65.4	67.3	69.2
Extrusion		76.4	83.3	84.8	84.9
P-value					
Starch source		0.383	0.590	0.411	0.643
Processing		<0.001	<0.001	<0.001	<0.001
Starch source x Processing		**<0.001**	**<0.001**	**<0.001**	**<0.001**

[1] Values are means of 10 replicate cages of 2 birds each.

[*] √MSE: square root of means square error in the analysis of variance.

[a, b, c, d] Means within column followed by different letters are significantly different ($P < 0.05$).

Table 9. *The effect of starch source and processing method on nitrogen disappearance rate (g/bird/day) along the intestinal tract of 30-d-old male broilers*[1]

		Jejunum		**Ileum**	
Starch source	**Processing**	Upper	Lower	Upper	Lower
W	Pelleting	2.8	4.4	4.7	6.0
FBS	Pelleting	2.2	4.0	5.3	6.0
W	Extrusion	1.9	4.0	5.4	6.1
FBS	Extrusion	1.9	4.4	5.5	6.3
	√MSE[*]	0.71	0.52	0.94	0.33
Starch source					
W		23	4.2	5.1	6.1
FBS		2.0	4.2	5.4	6.2
Processing					
Pelleting		2.5	4.2	5.0	6.0
Extrusion		1.9	4.2	5.5	6.2
P-value					
Starch source		0.278	0.903	0.326	0.253
Processing		**0.019**	0.964	0.167	0.095
Starch source x Processing		0.191	0.076	0.345	0.236

[1] Values are means of 10 replicate cages of 2 birds each.

[*] √MSE: square root of means square error in the analysis of variance.

Table 10. *The effect of starch source and processing method on the ratios of starch and nitrogen disappearance rates (**SNDR**) along the intestinal tract of 30-d-old male broilers*[1]

		Jejunum		Ileum	
Starch source	**Processing**	Upper	Lower	Upper	Lower
W	Pelleting	25.4	15.9	13.4 **b**	12.0 **b**
FBS	Pelleting	27.8	15.8	12.1 **c**	11.0 **c**
W	Extrusion	44.1	20.5	15.2 **a**	13.6 **a**
FBS	Extrusion	49.4	19.8	16.0 **a**	13.8 **a**
	√MSE*	15.86	2.04	0.90	0.43
Starch source					
W		35.8	18.3	14.4	12.8
FBS		39.2	17.8	14.1	12.4
Processing					
Pelleting		26.7	15.9	12.7	11.5
Extrusion		46.8	20.2	15.6	13.7
P-value					
Starch source		0.456	0.590	0.526	0.012
Processing		**<0.001**	**<0.001**	<0.001	<0.001
Starch source x Processing		0.781	0.665	**<0.001**	**<0.001**

[1] Values are means of 10 replicate cages of 2 birds each.

*√MSE: square root of means square error in the analysis of variance.

[a, b, c] Means within column followed by different letters are significantly different ($P < 0.05$).

Table 11. *The effect of starch source and processing method on the activities of digestive enzymes in the digesta collected from the lower jejunum*

Starch source	**Processing**	Amylase (U/g chyme)	Trypsin (U/g chyme)
W	Pelleting	64.7	4.1
FBS	Pelleting	82.9	3.1
W	Extrusion	54.8	4.8
FBS	Extrusion	77.1	3.9
	√MSE[*]	32.21	0.92
Starch source			
W		59.7	4.4
FBS		80.0	3.5
Processing			
Pelleting		73.8	3.6
Extrusion		66.0	4.3
P-value			
Starch source		**0.055**	**0.003**
Processing		0.444	**0.019**
Starch source x Processing		0.840	0.959

[1] Values are means of 10 replicate cages of 1 bird each

[*] √MSE: square root of means square error in the analysis of variance.

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Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no

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