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Ileal digestibility of amino acids for poultry

Doctoral Dissertation

Sini Perttilä

Luke

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Faculty of Agriculture and Forestry
University of Helsinki

Academic dissertation

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Abstract

The aim of this thesis was to determine the ileal amino acid digestibility values in the most commonly used feed ingredients for poultry in Finland. Four experiments were conducted to determine the apparent amino acid digestibilities of cereals (barley, oats, wheat, triticale and maize) and protein ingredients (rapeseeds, rapeseed cake, rapeseed meal, soybeans, soybean cake, soybean meal and meat and bone meal) for broilers (in publication I, II, III and IV) and adult cockerels (I). The flows of basal endogenous amino acids at the distal ileum of the broilers were determined using a protein-free diet (IV), and standardised ileal amino acid digestibilities were calculated for wheat, soybean meal and rapeseed meal (IV). Further, the effects of β-glucanase supplementation and preservation method of barley on apparent ileal amino acid digestibility (AID) in broilers and cockerels were determined (I). The ileal digestibility values were determined using the slaughter technique and chromium mordanted straw as an indigestible marker. Finally, apparent ileal digestible lysine based feed formulation was compared to total lysine based feed formulation for broilers (IV).

Lysine AID was lower in barley than in triticale and wheat, but did not differ from dehulled oats, oats and maize (II). Methionine AID was the lowest in barley, intermediate in oats, wheat and dehulled oats and highest in maize but maize did not differ significantly from triticale and dehulled oats. In addition, methionine AID in wheat did not differ from dehulled oats and triticale. Furthermore, cysteine AID was highest in wheat, triticale, maize and barleys and lowest in oats but oats did not differ from dehulled oats and maize. Threonine AID was similar among all the grains.

Among the protein ingredients, lysine AID was highest in soybean cake, soybean meal and full-fat soybeans, intermediate in rapeseed cake, rapeseed meal and meat and bone meal and lowest in rapeseeds but rapeseeds did not differ from meat and bone meal and rapeseed meal (III). Methionine AID was lowest in meat and bone meal and full-fat rapeseeds, intermediate in full-fat soybeans, rapeseed meal and rapeseed cake and highest in soybean meal and cake, but soybean meal and cake did not differ from rapeseed cake. Cysteine AID was lowest in meat and bone meal and rapeseed meal, intermediate in rapeseed cake and rapeseeds and highest in soybean cake, soybean meal and full-fat soybeans. Threonine AID increased from full-fat rapeseeds and meat and bone meal to rapeseed meal, further to rapeseed cake and onwards to full-fat soybeans, soybean meal and cake. Threonine AID in meat and bone meal did not differ from rapeseed meal, and rapeseed cake did not differ from full-fat soybeans.

The AID of cysteine and proline in dried barley containing diets were lower than in air-tightly stored and ensiled barley containing diets (I). The AID of alanine, glutamic acid, isoleucine, methionine, phenylalanine, and proline were lower in air-tightly stored barley compared to that in ensiled barley. The enzyme β-glucanase improved the AID of amino acids in the dried barley containing diet for broilers, but had no effect on the air-

tightly stored and ensiled barley containing diets for broilers, or on any diets for cockerels. Amino acid AID was lower in broilers than in cockerels.

The predominant basal endogenous amino acids were aspartic acid and glutamic acid and the lowest ones present were methionine and histidine (IV). Lysine and threonine standardised ileal digestibility (SID) was lower in rapeseed meal compared to that in soybean meal and wheat. Methionine SID was similar among soybean meal, rapeseed meal and wheat. Cysteine SID was much lower in soybean meal and rapeseed meal (21.8 and 21.4 %-units lower, respectively) than that in wheat.

Differences in amino acid digestibility values of tested ingredients could be explained by variation in chemical composition. Structure of protein fractions and fibre, non-starch polysaccharides and anti-nutritional factors affect digestibility of ingredients. Ingredients with higher fibre content such as cereals and rapeseed products had lower amino acid digestibilities than low fibre ingredients such as soybean meal.

The difference between amino acid AID and SID values were the highest in wheat and decreased from rapeseed to soybean meal. The results from growing experiment (III) imply that formulating diets based on a lysine AID was better than that based on total lysine when diets contained protein sources of low amino acid digestibility such as meat and bone meal and rapeseed meal. Variation in the difference between amino acid AID and SID values of different ingredients explains the need to standardise and use standardised amino acid SID values in feed formulation.

The amino acid digestibility values measured in current experiments could be added to Finnish feed tables. This would allow formulate more accurately the crude protein-feeding of broilers fed diets composed of different feed ingredients.

Keywords: broiler, cereal, protein ingredient, apparent, standardised, diet formulation

Aminohappojen ohutsuolisulavuus siipikarjalla

Tiivistelmä

Väitöskirjatyön tutkimusten tavoitteena oli määrittää tavallisimpien Suomessa siipikarjalle syötettävien rehuaineiden aminohappojen ohutsuolisulavuus. Yhteensä neljässä tutkimuksessa selvitettiin viljojen (ohra, kaura, vehnä, ruisvehnä ja maissi) sekä valkuaisrehujen (rypsin siemenet, rypsipuriste, rypsirouhe, soijapavut, soijapuriste, soijarouhe sekä liha- ja luujauho) näennäinen aminohappojen sulavuus (AID) broilereille (julkaisut I, II, III ja IV) ja aikuisille kukoille (I). Aminohappojen endogeeninen eritys määritettiin proteiinittoman dieetin avulla ohutsuolen loppuosasta ja sen avulla laskettiin vehnän, soijarouheen ja rypsirouheen aminohappojen standardoidut ohutsuolisulavuudet (IV). Lisäksi määritettiin β-glukanaasi-entsyymin ja ohran säilöntämenetelmän vaikutus aminohappojen näennäiseen ohutsuolisulavuuteen broilereilla ja kukoilla (I). Ohutsuolisulavuudet määritettiin näennäisesti teurastustekniikalla käyttäen kromiolkea sulamattomana merkkiaineena. Lopuksi ohutsuolisulavaa lysiiniä verrattiin kokonaislysiiniin rehuseoksen suunnittelun perustana broilereiden ruokinnassa (IV).

Lysiinin AID oli alhaisempi ohrassa kuin ruisvehnässä ja vehnässä, mutta ohran lysiinin AID ei eronnut kuoritun kauran, kauran ja maissin lysiinin AID:sta (II). Metioniinin AID oli alhaisin ohrassa, keskitasoa kaurassa, vehnässä ja kuoritussa kaurassa sekä korkein maississa, mutta maissin metioniinin AID ei eronnut kuitenkaan merkitsevästi ruisvehnästä ja kuoritusta kaurasta. Lisäksi metioniinin AID vehnässä ei eronnut kuoritusta kaurasta ja ruisvehnästä. Kysteiinin AID oli korkein vehnässä, ruisvehnässä, maississa ja ohrassa ja alhaisin kaurassa. Kauran kysteiinin AID ei eronnut kuoritusta kaurasta ja maissista. Treoniinin AID oli samanlainen kaikissa viljoissa. Valkuaisrehuissa lysiinin AID oli korkein soijapuristeessa, soijarouheessa ja soijapavuissa, keskitasoa rypsipuristeessa, rypsirouheessa ja lihaluujauhossa sekä alhaisin rypsin siemenissä (III). Rypsin siementen lysiinin AID ei eronnut lihaluujauhosta ja rypsirouheesta. Metioniin AID oli alhaisin lihaluujauhossa ja rypsin siemenissä, keskitasoa soijapavuissa, rypsirouheessa ja –puristeessa sekä korkein soijarouheessa ja –puristeessa. Soijarouheen ja -puristeen metioniin AID ei eronnut rypsipuristeesta. Kysteiinin AID oli alhaisin lihaluujauhossa ja rypsirouheessa, keskitasoa rypsipuristeessa ja rypsin siemenissä sekä korkein soijapuristeessa, soijarouheessa ja soijapavuissa. Treoniinin AID suureni rypsin siemenistä ja lihaluujauhosta rypsirouheeseen, rypsipuristeeseen ja edelleen soijapapuihin, soijarouheeseen ja soijapuristeeseen. Treoniinin AID lihaluujauhossa ei eronnut rypsirouheesta ja rypsipuristeen treoniinin AID soijapavuista.

Kuivattua ohraa sisältäneen rehuseoksen kysteiinin ja proliinin AID oli alhaisempi kuin ilmatiivisti säilöttyä tai murskesäilöttyä ohraa sisältäneiden rehuseosten (I). Alaniinin, glutamiinihapon, isoleusiinin, metioniinin, fenyylialaniinin ja proliinin AID oli alhaisempi ilmatiivisti säilötyssä ohrassa verrattuna murskesäilöttyyn ohraan. β-glukanaasi entsyymi paransi kuivattua ohraa sisältäneen rehuseoksen aminohappojen sulavuutta broilereille, mutta ei vaikuttanut ilmatiiviisti säilöttyä tai murskesäilöttyä ohraa sisältäneen rehuseoksen aminohappojen sulavuuteen broilereilla tai minkään rehuseoksen aminohappojen sulavuuteen kukoilla. Aminohappojen AID oli alhaisempi broilereilla kuin kukoilla.

Endogeeninen eritys sisälsi eniten asparagiinihappoa ja glutamiinihappoa ja vähiten metioniinia ja histidiiniä (IV). Rypsirouheen lysiinin ja treoniinin standardisoitu ohutsuolisulavuus (SID) oli alhaisempi kuin soijarouheen tai vehnän. Metioniinin SID oli samanlainen soijarouheessa, rypsirouheessa ja vehnässä. Soijarouheen ja rypsirouheen kysteiinin SID oli paljon alhaisempi kuin vehnän.

Erot testattujen rehuaineiden aminohappojen sulavuuksissa selittyvät osittain kemiallisen koostumuksen eroilla. Proteiinifraktioiden ja kuidun rakenne, ei-tärkkelyspolysakkaridien ja haitta-aineiden määrä sekä koostumus vaikuttavat rehuaineiden sulavuuteen. Rehuaineiden, joiden kuidun määrä on korkea, kuten viljojen ja rypsituotteiden, aminohappojen sulavuus on alhaisempi kuin vähän kuitua sisältävien rehuaineiden kuten soijarouheen.

Ero AID- ja SID-arvojen välillä oli suurin vehnässä ja väheni rypsirouheesta soijarouheeseen. Kasvatuskokeen (III) tulosten mukaan rehuseosten suunnittelu perustuen lysiinin näennäiseen sulavuuteen oli parempi tapa verrattuna kokonaislysiiniin perustuvaan rehusuunnitteluun, rehuseosten sisältäessä valkuais-rehuja, kuten lihaluujauho ja rypsirouhe, joiden aminohappojen sulavuus on alhainen. Koska AID- ja SID-arvojen välinen ero vaihteli eri rehuaineissa, on syytä käyttää aminohappojen SID arvoja rehujen suunnittelussa.

Väitöskirjan tutkimuksissa määritetyt aminohappojen sulavuusarvot voidaan liittää suomalaisiin rehutaulukoihin ja niiden avulla voidaan tarkentaa broilereiden valkuaisruokinnan suunnittelua ja toteutusta eri rehuaineista muodostuvilla rehuseoksilla.

Asiasanat: broileri, vilja, valkuaisrehu, näennäinen, standardoitu, rehusuunnittelu

“Fall down seven times, stand up eight.”
Japanese proverb.

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Mäntsälä 22 June 2020 Sini Perttilä

List of original publications

This thesis is based on the following publications referred to in the text by their Roman numerals I-IV:

I Perttilä. S., Valaja, J. Partanen, K., Jalava, T., Kiiskinen, T. and Palander, S. 2001. Effects of preservation method and β-glucanase supplementation on ileal amino acid digestibility and feeding value of barley for poultry. British Poultry Science 42: 218-229.

II Perttilä, S., Valaja, J. and Jalava, T. 2005. Apparent ileal digestibility of amino acids and metabolisable energy value in grains for broilers. Agricultural and Food Science 14: 325-334.

III Perttilä, S., Valaja, J., Partanen, K., Jalava, T. and Venäläinen, E. 2002. Apparent ileal digestibility of amino acids in protein feedstuffs and diet formulation based on total vs digestible lysine for poultry. Animal Feed Science and Technology 98: 203-218.

IV Perttilä, S., Jalava, T., Rinne, M., Da Silva Viana, G. and Valaja, J. 2019. Apparent and standardised ileal digestibilities of amino acids in wheat, soybean and rapeseed meals for broilers. Manuscript.

Contributions

The contributions of all authors to the original articles of this thesis are presented in the following table:

	I	*II*	*III*	*IV*
Planning the experiment	JV, KP, SP, TK	SP, JV	JV, KP, SP	SP, JV
Conducting the experiment	SP, JV, KP, TK	SP, JV	SP, JV, ET	SP, JV
Data analysis	SP, JV, TJ	SP, JV, TJ	SP, ET, JV, TJ	SP, JV, TJ
Calculating and interpreting the results	SP, JV, TJ	SP, JV, TJ	SP, ET, JV, TJ	SP, JV, TJ
Manuscript preparation	SP, JV, KP, TJ	SP, JV, TJ	SP, JV, TJ	SP, JV, MR, GSV

GSV = Gabriel Da Silva Viana
TJ = Taina Jalava
TK = Tuomo Kiiskinen
SP = Samu Palander
KP = Kirsi Partanen
SP = Sini Perttilä
MR = Marketta Rinne
JV = Jarmo Valaja
ET = Eija Venäläinen (currently Talvio)

Abbreviations

AA	amino acid
$AA_{Digesta}$	amino acid concentration in ileal digesta
AA_{Feed}	amino acid concentration in feed
AID	apparent ileal digestibility
AED	apparent excreta digestibility
AME	apparent metabolisable energy
AME_N	apparent nitrogen-corrected metabolisable energy
Basal $AA_{digesta}$	basal endogenous ileal amino acid secretion
Cr	chromic(III) oxide (Cr_2O_3)
$Cr_{Digesta}$	chromic(III) oxide (Cr_2O_3) in ileal digesta
Cr_{Feed}	chromic(III) oxide (Cr_2O_3) in feed
DM	dry matter
NDF	neutral detergent fibre
NSP	non-starch polysaccharide
SID	standardised ileal digestibility

Contents

1. Introduction

Broiler meat production and consumption has tripled since 1995 in Finland (Luke 2020b). The negative consequence of the intensified animal production is the increased phosphorus and nitrogen flow to the environment. One way to decrease nitrogen flow would be a more precise protein feeding. Digestible amino acid based feed formulation has been proven to decrease feed costs compared to feed formulation based on total amino acids in broiler diets (Rostagno et al. 1995, Maiorka et al. 2004, Dari et al. 2005). In addition, previously the digestibility was measured from the excreta content although amino acids are not absorbed after the ileum and hindgut microbes modify the amino acid content of lower digestive tract resulting in erroneous digestibility values when measured from excreta (Ravindran et al 1999, Kadim et al. 2002). Large differences between the ileal and the excreta digestibility coefficients were determined for several feed ingredients and the ileal amino acid digestibility should be preferred (ten Doeschate et al. 1993, Ravindran et al. 1999).

Protein feeding of poultry can be even further refined. Apparent ileal amino acid digestibility (AID) does not account for the amino acids of endogenous origin and therefore, the digestibilities of feed amino acids are underestimated if that is not taken into account (Lemme et al. 2004). Lemme et al. (2004) divided the endogenous amino acid losses into a basal (or non-specific) and a feed specific fraction. Standardised ileal digestibility (SID) values of amino acids are corrected for the basal endogenous amino acid losses and the term “true ileal digestibility” is used when the digestibility values are corrected for both the basal and the diet-specific losses (Lemme et al. 2004). Amino acid SID values were noticed to be more additive than AID when they were used for calculating the digestibility of a complete diet in feed formulation (Kong and Adeola 2013).

The basal endogenous losses can be measured with protein-free diet (Siriwan et al. 1993, Ravindran et al. 2004), feeding highly digestible protein (Adedokun et al. 2007a), or with the regression method (Short et al. 1999, Eklund et al. 2008). Adedokun et al. (2011) noticed that the protein-free diet seems to be the most consistent and reliable method for estimating the endogenous amino acid flow. However, Adedokun et al. (2011) noticed that because of large variation in used material and methods of digestibility assays, the basal ileal endogenous amino acid flow should be determined every time simultaneously in studies where the standardised digestibilities are determined.

Within-method variation is also large and the basal endogenous secretions, and therefore also digestibility values, vary a lot between experiments and are not directly comparable. Endogenous secretions and amino acid digestibilities are noticed to be affected by the age of the birds; the digestibility values are higher and the endogenous secretions lower in older animals (Adedokun et al. 2007b, Szczurek 2009 and

2010, Toghyani et al. 2015). In addition, endogenous secretions and digestibility values vary depending on the strains and the species of the birds (Huang et al. 2006, Adedokun et al. 2007a, Adedokun et al. 2014).

Furthermore, the digesta collection method may release epithelial cells, mucus and mucin from the intestinal wall to the digesta, thus increasing the amount and concentration of the mucosal and mucus amino acids in endogenous secretions (Poureslami et al. 2012). However, the digestive section (terminal or entire ileum) for collection of digesta did not affect the apparent ileal amino acid digestibilities in broilers according to Adedokun et al. (2011) and Poureslami et al. (2012).

Kadim et al. (2002) suspected that the composition of tested ingredients may have an effect on digestibility of basal diet, and therefore measured digestibility values are not additive. Anti-nutritive factors within the feed ingredients may decrease the digestibility values by increasing enzyme production and formation of indigestible complexes (Angkanaporn et al. 1994). However, Gallardo et al. (2017) noticed that with dietary combination of phytase and carbohydrase, amino acid digestibility can be increased. According to Dänicke et al. (1999), enzyme supplementation decreases the mean retention time of digesta by increasing gut motility, decreasing digesta viscosity and microbial fermentation. Therefore, barley-based diets for broilers are commonly supplemented with β-glucanase. However, high-moisture storage has reduced the β-glucan content of barley and because of the β-glucan degradation during ensiling, the amino acid digestibility in barley is suspected to be improved by ensiling (Svihus et al. 1995).

Negative effects of thermal processing of ingredients on nutrient digestibility are well known (Newkirk et al. 2003ab, Kasprzak et al. 2016, 2017), but also plant genotype, variety and origin have induced variation in amino acid digestibility of feed ingredients (Cowieson 2005, Coca-Sinova et al. 2008, Frikha et al. 2012, Ravindran et al. 2014, Zuber et al. 2017).

Frikha et al. (2012) noticed positive correlation between crude protein content and SID of lysine in soybean meals. In previous experiments, high dietary protein concentration has increased amino acid digestibility but also the endogenous secretion through increased enzyme production (Green et al. 1987, Ravindran et al. 2004, Adedokun et al. 2007b, Golian et al. 2008, Tahir and Pesti 2012). In addition, dietary fibre is suspected to change the viscosity and the passage rate of the ileal digesta influencing the secretion of mucin and epithelial cell turnover which results in decreasing amino acid digestibilities (Adedokun et al. 2011, Adeola et al. 2016).

The demand to decrease feed costs and increase profitability of the production has raised interest to use more by-products and other underutilized protein sources in broiler diets. Unfortunately, these products often have low digestibility. The difference between the AID and the SID of amino acids is noticed to vary according to the ingredient, the difference being larger for low digestibility and high fibre ingredients

(Lemme et al. 2004, Hoehler et al. 2006). When by-products and feed ingredients with low amino acid digestibility, such as wheat bran, feather meal and meat and bone meal (banned since 1996) are used in diets formulated based on digestible amino acids instead of total amino acids, the feed conversion and feed efficiency have increased. At the same time, feed costs have decreased and profitability of production increased (Rostagno et al. 1995, Maiorka et al. 2004, Dari et al. 2005). As a conclusion, by-products and feed ingredients with low amino acid digestibility can be used more efficiently in poultry feeding when SID of amino acids are used in diet formulation.

During the last decade, several experiments determining standardised ileal amino acid digestibility values for poultry have been published and amino acid SID values for soybean meal (Adedokun et al. 2009a, Szczurek 2009, Frikha et al. 2012, Kong and Adeola 2013, Ravindran et al. 2017,) for rapeseed meal (Adedokun et al. 2009a, Szczurek 2009, Woyengo et al. 2010, Tokhyani et al. 2015, Gallardo et al. 2017, Kasprzak et al. 2016, Osho et al. 2019) and for wheat (Szczurek 2009, Bandegan et al. 2011, Osho et al. 2019) are available. However, values for the ileal amino acid digestibility for the ingredients commonly used in Nordic countries, such as fibre-rich barley, oats and rapeseed products, are scarce in literature. In addition, chemical composition of previously tested ingredients varies a lot and there is large variation in the terminology and the methodology used. Therefore, additional experiments should be done to obtain accurate amino acid digestibility values, which are additive and suitable to be used in formulating poultry diets based on typical Finnish feed ingredients.

2. Aims of the study

The objective of this thesis was to evaluate the apparent and standardised ileal digestibility values of amino acids in the most commonly used feed ingredients for broilers and thus to establish a more precise basis for diet composition in feed formulation.

More specifically, the experiments were conducted:

1. To evaluate the apparent ileal amino acid digestibility in soybean (*Glycine max*) meal and cake, rapeseed (*Brassica napus, Brassica rapa*) meal and cake, meat and bone meal, full-fat soybeans, full-fat rapeseeds, barley (*Hordeum sativum*), oats (*Avena sativa*), wheat (*Triticum aestivum*), triticale (*Triticale*) and maize (*Zea mays*).
2. To evaluate the effect of different barley preservation methods: drying, airtight storage of whole grains, or ensiling rolled high-moisture grain, with β-glucanase enzyme supplementation on amino acid digestibility of barley for broilers and adult cockerels.
3. To compare a diet formulation system based on the apparent ileal digestible amino acids to the total amino acids based system, and the effects of the systems on the diet protein utilisation and nitrogen excretion.
4. To evaluate basal endogenous secretion of amino acids in broilers.
5. To investigate the standardised ileal digestibility values of amino acids in barley, soybean meal and rapeseed meal and investigate the differences between the apparent and the standardised amino acid digestibility values.
6. To evaluate the effects of animal age on amino acid digestibility.

The main hypotheses tested in this research, were:

1. The apparent and standardised amino acid digestibility values in most feed ingredients used in poultry production in Finland differ from each other and the reason for that are differences in the chemical composition of the ingredients.
2. Apparent ileal digestible amino acids based feed formulation system decreases nitrogen excretion and increases broiler performance results compared to a system based on total amino acids.
3. Standardised ileal amino acid digestibility values in feed ingredients can be calculated from apparent values by subtracting endogenous secretions, and the difference between AID and SID increases with increasing amino acid concentration and decreasing nutrient digestibility of the feed ingredients.

3. Materials and methods

All five experiments were conducted at MTT Agrifood Research Finland in Jokioinen (Natural Resources Institute Finland (Luke) since 2015). The experimental arrangements including animals, diets, experimental procedures, chemical analyses conducted, formulas used in calculations and statistical analyses were reported in detail in the original publications (I-IV). A general description of the main design, analyses and the measured parameters of each experiment is presented briefly in this section (Table 1 and Table 2). All the studies were approved by the Local or National Ethical Committee for Animal Experiments.

3.1. Animals, housing and management

One-day-old 308-Ross broiler chickens were obtained from a hatchery and raised in experimental battery wire cages (47.5 × 56 × 40 cm) with five birds (I and II) or four birds (III and IV) per cage. In digestibility experiments I and II at the last day of adaptation period, one broiler from each cage was humanely killed for ileal digesta viscosity determination and four broilers in each cage continued to the end of the experiment. Equal numbers of both sexes per treatment were used for excreta and digesta collections and digesta viscosity determination. In I adult Leghorn cockerels were housed in digestibility battery wire cages using two cockerels per cage. In the growing experiment (III), birds were obtained one-day-old from a hatchery and raised in peat litter floor pens (2 m × 2 m), 60 females or 60 males in each. The temperature, light, relative humidity and ventilation in the broiler houses were controlled according to Ross broiler Management Handbook (www.aviagen.com). Feed and water were available *ad libitum* with the exception of the 24 hours of fasting before ileal digestibility determination in digestibility experiments (I-IV).

3.2. Experimental designs and procedures

The cages were randomly allocated to the experimental treatments and cage was an experimental unit. Cockerels (I) were divided into equal blocks according to their liveweight and within the block cages of 2 birds were randomly allocated to the treatments.

Every digestibility experiment consisted of 3-5-days adaptation, 3-days excreta collection, 2-4-days adaptation and 24-h fasting periods, 4-h free access to feed and collection of ileal digesta. Experiment IV consisted only of 3-day adaptation period, 24-h fasting, 4-h free access to feed and collection of ileal digesta. Protein-free diet was fed to four cages of four broilers at the last day of second adaptation period and 4 h before ileal digesta collection in IV. After the adaptation period, one bird per cage was killed for digesta viscosity determinations in experiments I and II. Digesta were collected with the slaughter method. Birds were killed by stunning with carbon dioxide and subsequent cervical dislocation.

3.3. Tested ingredients and diets

Barley (Hordeum vulgare 'Inari') used as ensiled barley (I) was harvested at the dough ripeness stage, roller milled and preserved with formic acid, stored in pilot scale silos, and compressed. For air-tight storage and dried barley (I), barley was harvested at full ripeness state. For air-tight storage, whole grains were stored in closed plastic bags within plastic cans without a preservative. Dried barley was dried with warm air. Both air-tightly stored and dried barley were roller-milled before incorporation into the diets. Protein ingredients were obtained from commercial suppliers.

Broilers were fed with standard broiler starter and/or grower diets before digestibility trials. In I broilers were fed with standard broiler starter and grower diets (220 and 200 g kg^{-1} crude protein, respectively) from day 1 to day 14 and from day 15 to day 20, respectively. In II and III broilers were fed with standard starter diet from day 1 to day 23 and in IV standard starter diet from day 1 to day 14 grower diets from day 15 to day 31. In the performance experiment (III) broilers received experimental diets from day 1 to day 36.

In I, test diets contained 500 g of each barley tested and 500 g of basal diet on a dry matter basis. The semi-purified experimental diets in II, III and IV were composed of minerals, vitamins and the tested ingredient as the sole source of protein. Diets were formulated to be isoenergetic by varying amounts of starch, cellulose and vegetable oil and also to meet Finnish nutrient requirements for broilers (Luke 2020a). In the growing experiment (III), the diets were formulated to meet either total lysine or digestible lysine requirements of growing broilers. Diets contained a fixed amount of barley, rapeseed oil, minerals and vitamins and soybean meal, rapeseed meal and meat and bone meal alone or in combinations. Contents of digestible methionine and threonine were adjusted to meet requirements. Values for AID lysine, methionine and threonine in barley were derived from measurements from II. In IV the protein-free diet consisted of minerals, vitamins, rapeseed oil, glucose, starch and cellulose. In all digestibility experiments chromium mordanted straw (0.2 g Cr kg^{-1} feed) was used as an indigestible marker. In II, III and IV the grains were roller-milled with a 4.0 mm sieve before dietary incorporation. Test ingredients were mixed in a batch mixer and cold-pelleted into 4-mm pellets (Amandus Kahl Laborpresse L175, Reinbek, Germany).

3.4. Chemical analyses and data collection

Ingredient samples were taken before preparation of the experimental diets, and from the experimental diets prior to feeding. Excreta were collected daily from plates under the cages and stored frozen prior to analysis. Digesta was collected from the distal half of the ileum (distal half from Meckel's diverticulum to the ileo-caeco-colic junction). The digesta of birds within a cage was pooled before analysis. Birds were weighed at the beginning and end of the experiments and at the beginning and end of excreta collection. In the growing experiment (III), broilers were weighed at the beginning, when 14-day-old and at the end of the experiment. The feed intake was recorded during the collection period and the 4-h pre-slaughter feeding period from each cage and between weighing in growing experiment (III). At the beginning, four one-day old broilers (growing experiment III) were slaughtered in order to measure carcass nutrient retention during the experiment. At the end of experiment III, two broilers from each cage were humanely killed to measure the mass of breast muscles and abdominal fat. In addition, two broilers were humanely killed to measure fat, protein and ash retention to the body. Carcasses were stored frozen and ground in groups of two using a meat grinder. The mortality was recorded daily in all experiments and samples from the litter were taken in the growing experiment (III) at the 14-day old stage and at the end of the experiment. The ileal digesta samples and air-tightly stored and ensiled barley samples were freeze-dried and the excreta samples were dried overnight at 60°C. The feed samples were passed through a hammer mill fitted with a 1-mm mesh prior to analysis. All analyses were performed in duplicate unless otherwise stated. Detailed information of individual analytical procedures is described in the relevant papers (I-IV).

3.5. Calculations and statistical analyses

The digestibilities of test barleys in I were calculated by difference using measured digestibility coefficients for the basal diet and for the complete diets, containing the experimental ingredients and basal diet. In digestibility experiments II, III and IV, the direct method and semi-purified diets were used. In IV, the amino acid composition of endogenous secretion was determined with protein-free diet and amino acids SID were calculated.

Apparent ileal (AID) and excreta (AED) digestibilities were calculated using chromic(III) oxide (Cr_2O_3) as an indigestible marker as follows:

$$\text{AID, \%} = [1-(CR_{Feed}/CR_{Digesta}) \times (AA_{Digesta}/AA_{Feed})] \times 100$$

where Cr_{Feed} is the chromium content in feed (mg kg^{-1} DM), $Cr_{Digesta}$ chromium content in digesta (mg kg^{-1} DM), $AA_{Digesta}$ amino acid content in digesta (g kg^{-1} DM) and AA_{Feed} is the amino acid content in feed (g kg^{-1} DM).

Standardised ileal digestibilities were calculated for apparent ones as follows (Adeola et al. 2016):

$$\text{SID, \%} = \text{AID} + (\text{basal } AA_{Digesta} / AA_{Feed}) \times 100$$

where basal $AA_{Digesta}$ is the amino acid flow in nitrogen-free diet (g kg^{-1} DM) and calculated as follows (Adeola et al. 2016):

$$\text{basal } AA_{Digesta} = AA_{Digesta} \times (Cr_{Feed}/Cr_{Digesta})$$

Apparent metabolisable energy values (AME) were calculated by subtracting the gross energy (GE) of excreta from GE intake and then dividing by total dry matter intake. The AME values of diets were corrected for zero nitrogen retention (AME_n) by assuming a value of 36.55 kJ g^{-1} of nitrogen lost or retained (Titus et al. 1959).

Statistical analyses of experimental data form digestibility experiments were performed with the GLM procedure of the SAS (SAS Institute Inc., Cary, NC, USA). Experimental unit was a cage. Differences were considered to be significant at $P < 0.05$. Digestibility experiments were subjected to analysis of variance using a model which included the effects of diet and block. Performance experiment (III) was also subjected to analysis of variance using a model which included effects of diet, sex and sex × diet interaction. Significant differences between dietary treatments were tested by orthogonal contrasts (I and growing experiment III) or Tukey -tests (digestibility experiments II, III and IV). When comparing digestibilities of broilers and cockerels (I), a model which included diet, trial and trial × diet was used.

Table 1. Design of the experiments.

Publ.	*Treatments*	*Animals*	*Replicates/treatment*	*Animals/replicate*	*Age*
I	4	145 Ross 308-broilers and 32 Leghorn cockerels	4 (and 5 per basal diet)	Broilers 5 until d 25 / 4 after d 25 and cockerels 5 until d 24 / 4 after d 24	Broilers 1–33 d and cockerels 96–109 d
II	6	180 Ross 308-broilers	6	5 until d 25 / 4 after d 25	1–34 d
III	7	140 Ross 308-broilers	5	4	1–35 d
III	6	2880 Ross 308-broilers	8	60	1–37 d
IV	4	64 Ross 308-broilers	4	4	1 – 36 d

Table 2. Treatments design, tested ingredients, diets, methods and objective of experiments.

Publ.	*Design*	*Tested ingredients*	*Diets/Methods*	*Objective*
I	7 diets	Dried, air-tightly stored and ensiled barley with or without β-glucanase enzyme and basal diet	Basal diet 50 % + barley 50 % on DM basis +/- β-glucanase difference-method	Preservation of barley and β-glucanase enzyme supplementation for broilers and cockerels, effect of age on amino acid AID in dried, air-tight stored and ensiled barley
II	6 diets	Oats, wheat, barley, triticale, maize, dehulled oats	Semi-purified diets, direct-method	Amino acid AID in grains
III	7 diets	Full-fat soybeans, soybean meal, soybean cake, full-fat rapeseeds, rapeseed meal, rapeseed cake, meat and bone meal	Semi-purified diets, direct-method	Amino acid AID in protein ingredients
III	6 diets	Basal diet + soybean meal, soybean meal + rapeseed meal or soybean meal+ rape-seed meal + meat and bone meal	3 diets total- and 3 diets AID lysine-based formulation	Amino acid AID vs. total amino acid in feed formulation
IV	4 diets	Wheat, soybean meal, rape-seed meal + protein-free	Semi-purified diets, direct-method, protein-free-diet	Amino acid AID, amino acid flow, amino acid SID in wheat, soybean meal and rape-seed meal, basal endogenous flow of amino acids

AID = apparent ileal digestibility
SID = standardized ileal digestibility

4. Results and discussion

4.1. Chemical composition of the ingredients

4.1.1. Grains

The chemical composition varied between the grains, but also the within ingredient variation was great (Table 3). The protein content of grains is known to vary greatly between cereals, cultivars (Siegert et al. 2017) and in different growing and environmental conditions (Gutierrez-Alamo et al. 2008). The crude protein content of the grains (I, II, IV) was lowest in maize and triticale and intermediate in wheat, oats and barleys, following the results of Rodehutscord et al. (2016), where crude protein content (in g kg^{-1} dry matter) ranged from 108 to 136 in barley, from 121 to 162 in wheat, from 113 to 138 in triticale, from 78 to 112 in maize and from 121 to 141 in oats. Dehulled oats in the current experiment had the highest crude protein content; over 17 %-units higher than in oats. This was slightly higher than in Biel et al. (2014) who reported that dehulled oats contained on average 137 g and hulled oats 105 g crude protein kg^{-1} dry matter. However, crude fibre content of barley, oats and triticale in II were within the range of Biel et al. (2014). Crude fibre content of maize and wheat in II and IV were slightly higher than those in Biel et al. (2014).

The crude protein content was from 3 to 4 g and total amino acid content 15 g kg^{-1} dry matter lower in ensiled than in dried or air-tightly stored barley (Table 3). In particular, arginine and lysine contents were lower in ensiled barley. Barley samples were harvested at different stage of ripeness (I). According to Schipper (1984), barley crude protein and amino acid content increased with maturity. Furthermore, Svihus, et al. (1997a) and Svihus, et al. (1997b) noticed that amino acid degradation during the preservation can be as high as 11 to 12 % at yellow ripeness stage of grain, but at the full ripeness stage, the degradation is less than 4 %, reflecting the differences in crude protein and amino acid content of barley preserved in different ways. In addition, β-glucan content of ensiled barley was very low compared to the other barleys, reflecting the stage of maturity (Åman et al. 1989). Further, according to Åman et al. (1989), intrinsic β-glucanase becomes active during ensiling and β-glucans can be degraded by microbial enzymes and hydrolysed by organic acids to sucrose and raffinose during preservation (Hesselman et al. 1981). This might be the reason for the higher sugar content of the ensiled barley in the current experiment.

In general, maize protein and amino acid content has been shown to vary greatly and it has relatively low crude protein content compared to wheat and barley (Cowieson 2005), which corresponds with the current results. Major storage proteins in maize are zein (occurring in the endosperm) and glutelin (occurring in the endosperm and in the germ). Zein is deficient in the indispensable amino acids tryptohan and lysine and amino acid content of glutelin is also nutritionally poor (Cowieson 2005). In II, leucine and alanine content of maize was clearly higher and glutamic acid and proline content lower than in the other cereals. However, amino acid content in maize was similar as in Ravindran et al. (1999).

The most important proteins in wheat endosperm are prolamin and glutelin and the proportion of glutamic acid and proline in wheat storage protein is high whereas content of lysine is very low (Gutierrez-Alamo et al. 2008), which explains the low content of lysine in the wheats compared to other cereals in current experiments. However, amino acid content in wheats confirmed previous results of McCracken and Quintin (2000) and Cave (1988). Bandegan et al. (2011) noticed that contents of methionine, cysteine and histidine were the lowest and contents of glutamic acid, proline and leucine the highest in both wheat and barley similarly as in NRC (1994) values, which correspond well with current results.

The amino acid content of the two wheat samples (II and IV) varied slightly, although their protein contents were rather similar; arginine, glutamic acid and threonine being slightly lower and valine slightly higher in the wheat in IV compared to the wheat in II. In general, an increase in wheat protein content is accompanied by a relative increase in storage proteins (glutens) rather than albumins and globulins and the former group have relatively more of the nutritionally non-essential amino acids (Mossé et al. 1985).Therefore the proportion of individual amino acids changes in wheat as its protein content increases. In addition, Bandegan et al. (2011) found variation from 7.5 to 12.0 %-units in the content of individual amino acids as the protein content varied from 14.8 to 18.4 % among different wheat samples. Most variable indispensable amino acids were isoleucine and histidine (Bandegan et al. 2011). In current experiments, the crude protein content of the wheat samples varied only slightly (142 vs 147 g kg^{-1} dry matter in II and V, respectively) which may explain the minor, but noticeable variation in the content of the above mentioned amino acids.

Amino acid contents of dried barley in I were within the range of Bandegan et al. (2011), but barley used in II had lower content of all amino acids. In addition, barley samples (I and II) had a greater difference in crude protein content (125 vs 132 g kg^{-1} dry matter) than wheat samples (II and IV), causing more variation in the amino acid content of samples, which correspond to the results of Bandegan et al. (2011) who noticed that the variation in the amino acid content in barley was more accompanied to variation in protein content than wheat amino acid content.

Triticale is a hybrid derived from crossing of wheat and rye. Its protein content is between wheat and barley (Rodehutscord et al. 2016) but the protein quality is better than that in wheat because of its higher proportion of lysine and sulphur-containing amino acids (Siegert et al. 2017, Zuber et al. 2017) as was seen in II. The amino acid content of the triticale in II was rather similar to that in Flores et al. (1994), except that serine, alanine, leucine and histidine contents were lower. In addition, Rundgren (1988) observed much lower amino acid content in triticale than in II. Siegert et al. (2017) noticed that the chemical composition, including the crude protein and the amino acid content, of the triticale varies according to the genotype and fertilisation and reported amino acid composition where the contents of most amino acids were slightly lower than in II.

Oats had the highest crude fibre content of the cereals (II). In general, the nutritive value of oats depends on the proportion of kernel to hull (Biel et al. 2014). Biel et al. (2014) showed that the removal of hulls from oats grains significantly increased the proportion of fat in oats and for the dehulled oats the fibre content was comparable with wheat, maize and barley, which corresponds to the current results. However, although dehulling increased crude protein content of dehulled oats in Biel et al. (2014), the crude protein content in their experiment, was remarkably lower compared to II. Major storage protein in oats is globulin (Klose and Arendt 2012) and it has lower content of cysteine and threonine, but the lysine content of it is slightly higher than in barley and wheat (Luke 2020a), which corresponds well with II. In general, dehulled oats has higher content of essential amino acids than oats (Luke 2020a). However, in II oats and dehulled oats had almost similar content of amino acids although their crude protein content differed. According to Peltonen-Sainio et al. (2004) dehulling improves nutritional value of oats, but dehulling-techniques can vary a lot resulting in variation in nutritive value of dehulled oats.

Table 3. Chemical composition of tested grains (g kg^{-1} dry matter (DM), unless otherwise stated).

Ingredient	Dried barley	Air-tight stored barley	Ensiled barley	Barley	Wheat	Wheat	Oats	Triticale	Maize	Dehulled oats
Publication		I		II		IV		II		
DM, g kg^{-1}	895	768	545	881	890	894	891	869	857	876
Crude protein	125	123	120	132	142	147	131	119	92	159
Crude fat	28	28	28	30	31	19	80	24	55	82
Crude fibre	48	50	52	48	32	29	100	26	26	45
Ash	29	30	31	23	17	18	30	17	12	23
Starch	495	531	525	630	659		495	686	749	621
β-Glucans	46	45	9	42	8		40	8		49
Soluble				36	6		40	6		43
Insoluble				6	2		0	2		6
Amino acids, g 16g^{-1} N										
Arginine	6.4	6.4	4.5	5.5	5.0	4.6	6.8	5.5	4.9	6.8
Histidine	3.2	3.1	2.9	2.4	2.5	2.5	2.3	2.4	3.2	2.4
Isoleucine	4.2	4.2	4.0	3.5	3.5	3.7	3.7	3.4	3.7	3.7
Leucine	8.3	8.2	7.8	6.9	6.9	6.8	7.2	6.6	12.9	7.5
Lysine	4.3	4.3	2.7	3.8	2.8	2.8	4.1	3.6	3.0	4.0
Methionine	2.1	2.1	2.1	1.8	1.9	1.6	1.7	2.0	2.4	1.9
Phenylalanine	6.0	6.0	5.7	5.0	4.5	4.9	4.9	4.7	4.8	5.1
Threonine	4.2	4.2	3.9	3.9	3.3	2.8	3.8	3.7	4.2	3.6
Valine	6.1	6.5	6.2	5.9	5.9	6.7	6.0	5.8	6.1	6.0
Alanine	5.0	5.1	5.2	3.9	3.6	3.5	4.5	3.9	7.5	4.4
Aspartic acid	7.5	7.4	5.1	6.3	5.2	4.9	8.0	6.2	6.6	7.8
Cystine	3.2	3.1	3.0	2.5	2.5	2.4	3.3	2.5	2.9	3.3
Glutamic acid	34.2	32.4	29.0	21.2	30.3	26.7	17.1	24.4	15.8	18.5
Glycine	5.0	5.0	4.7	3.9	3.9	4.1	4.7	4.1	3.7	4.5
Proline	12.0	12.3	11.1	10.7	10.8	10.7	5.2	9.2	9.1	5.3
Serine	5.3	5.2	4.6	4.4	4.7	4.7	4.8	4.9	5.2	4.7
Tyrosine	4.1	4.0	3.5	3.4	3.2	3.2	3.6	3.0	4.3	3.7

4.1.2. Protein ingredients

For the protein ingredients used in III and IV, the crude protein content of the meat and bone meal was the highest and that of rapeseeds the lowest (Table 4). The chemical content of meat and bone meal is attributed to differences in used raw material sources (meat and bone content) and the use of different processing techniques (Ravindran et al. 2002). High ash content in meat and bone meal indicates high bone content, which decreases nutrient digestibility (Ravindran et al. 2002). According to Ravindran et al. (2002), meat and bone meal contains from 390 to 670 g kg^{-1} crude protein and its amino acid content varies substantially. In III, the meat and bone meal crude protein content corresponded to that mentioned above (Ravindran et al. 2002), but lysine content of it was the lowest among the tested protein ingredients. However, the amino acid contents in the meat and bone meal corresponded well with results of Green and Kiener (1989). The concentrations of all indispensable amino acids were lower in the meat and bone meal compared to the other protein ingredients, except the content of arginine, which was similar in the meat and bone meal and in the soybean products (III and IV).

In current experiments, the rapeseed products had on average 22 %-units lower crude protein content than the soybean products. In addition, the rapeseed protein content (241 g kg^{-1} DM) was little over half of that of the full-fat soybeans (428 g kg^{-1} DM). While the amino acid profile was similar in the soybean products, corresponding with results of Frikha et al. (2012), their crude protein content varied from 428 g kg^{-1} dry matter for the full-fat soybeans to 450 g kg^{-1} dry matter for the soybean cake and to 496 g kg^{-1} dry matter for the soybean meal. Similarly, the rapeseed products had an amino acid content corresponding with results of Osho et al. (2019), but the crude protein content increased and fat content decreased from the rapeseeds to the rapeseed cake and meal. Lysine content was higher in the soybean based compared to the rapeseed based products, but the rapeseed products contained more sulphur-containing amino acids (methionine and cysteine) than the soybean products, which corresponded with results of Khajali and Slominski (2012). However, lysine content of the rapeseed meal corresponded well to Newkirk et al. (2003b), who determined the level of amino acids in 26 non-toasted and 31 toasted rapeseed meals and reported that the lysine content varied from 5.3 to 6.3 g per 16 g nitrogen. The crude fibre content in rapeseed meal was higher (133 g kg^{-1}dry matter) in the current experiment compared to tested rapeseed meals in Toghyani et al. (2015) where crude fibre content ranged from 104 to 122 g kg^{-1} dry matter. However, the crude fibre content in soybean meal (III) was 59 g kg^{-1} dry matter in agreement with the 37–76 g kg^{-1}dry matter in soybean meals tested by Frikha et al. (2012).

In general, soybean and rapeseed meals and cakes are by-products of oil industry. Oil is extracted by solvent or expeller extraction methods, using high moisture (steam) (expeller method) and/or high temperatures (solvent method) to obtain oil (Toghyani et al. 2015). Because soybeans contain anti-nutritive factors such as protease inhibitors and rapeseeds contain tannins and glucosinolates, soybean and rapeseed products are also heat-treated (Newkirk et al. 2003a, Newkirk et al. 2003b, Ravindran et al. 2014, Kasprzak et al. 2016, Kasprzak et al. 2017), although the content of anti-nutritive factors has also been decreased by plant breeding. Unfortunately, processing of the soya and the rapeseed products may change the nitrogen and the amino acid content and availability by altering the protein structure and producing amino acids damaged during heating (Newkirk et al. 2003a, Szczurek 2010, Toghyani et al. 2015). In addition, Frikha et al. (2012) concluded that differences between the soybean products are attributed to the growing conditions and the geographical location, the processing such as the heat-processing and the steaming and therefore, the nutritional value of the soybean products are much more variable than previously believed. The used raw material, temperature, pressure and heating time during the processing affects the composition and the digestibility of the ingredients. Detailed processing of the tested protein ingredients in current experiment is presented in III.

Table 4. Chemical composition of tested protein ingredients (g kg^{-1} dry matter (DM), unless otherwise stated).

Ingredient	Meat and bone meal	Full-fat soy-beans	Soy-bean cake	Soybean meal	Soy-bean meal	Rape-seeds	Rapeseed cake	Rape-seed meal	Rape-seed meal
Publication	III				IV	III			IV
DM, g kg^{-1}	977	918	892	888	894	929	894	873	900
Crude protein	531	428	449	496	470	241	360	391	357
Crude fat	155	244	96	45	33	441	108	42	41
Crude fibre	25	30	64	57	59	198	133	136	133
Ash	289	51	57	61	60	49	68	79	75
Amino acids, g 16 g^{-1} N									
Arginine	7.3	8.2	7.5	7.7	7.1	6.3	6.2	6.3	6.0
Histidine	1.8	3.1	3.0	3.0	2.6	3.1	2.9	3.0	2.7
Isoleucine	2.2	4.0	4.1	4.2	4.5	3.9	3.5	3.6	4.0
Leucine	5.9	8.5	8.2	8.2	7.8	7.2	7.3	7.6	7.2
Lysine	4.3	6.5	6.2	6.5	6.2	6.2	4.9	5.5	5.6
Methionine	1.0	1.2	1.2	1.2	1.4	2.0	1.9	1.9	2.1
Phenylalanine	3.3	5.6	5.4	5.4	5.1	4.2	4.3	4.3	4.1
Threonine	3.1	3.8	4.1	4.1	3.8	4.6	4.8	4.8	4.5
Valine	3.0	4.4	4.3	4.0	5.3	4.5	4.5	4.5	5.9
Alanine	7.5	4.6	4.4	4.4	4.3	4.6	4.6	4.6	4.5
Aspartic acid	6.7	11.0	11.0	11.1	10.9	7.8	7.4	7.4	7.4
Cystine	0.7	1.6	1.6	1.7	1.5	2.5	2.2	2.4	2.0
Glutamic acid	15.0	21.2	19.9	21.8	18.0	18.5	18.3	18.8	14.3
Glycine	11.0	4.3	4.5	4.4	4.1	5.4	5.3	5.3	5.1
Proline	7.3	5.1	5.0	4.9	5.0	5.5	5.6	5.6	6.3
Serine	3.8	5.1	5.4	5.3	5.0	4.5	4.7	4.6	4.3
Tyrosine	2.3	3.9	3.9	4.0	3.7	3.2	3.2	3.3	3.2

4.2. Apparent ileal amino acid digestibility

4.2.1. Grains

The mean amino acid AID was the highest in wheat and triticale, intermediate in maize and dehulled oats and the lowest in barley and oats (II, IV) (Table 5). Lysine AID was lower in barley than in triticale and wheat, but did not differ from dehulled oats, oats and maize. Methionine AID was the highest in maize but did not differ from triticale and dehulled oats. Furthermore cysteine AID was the highest in wheat, triticale, maize and barleys, the lowest in oats and did not differ in dehulled oats and maize. Threonine AID was similar among all the grains (II).

Wheat essential amino acids AID in II and IV in comparison to literature values is presented in Figure 1. Mean amino acid AID in wheat in IV was 4 %-units lower than in II although the crude protein content was only slightly higher in the wheat in IV than in II (147 vs 142 g kg^{-1} DM). However, cysteine AID was similar between the current wheats. In addition, the AID of essential amino acids in the wheat in II were higher compared to Bandegan et al. (2011), except for cysteine and methionine which were higher and phenylalanine which was similar. The AID of essential amino acids in IV corresponded well with the results of Bandegan et al. (2011), except for cysteine AID which was lower and lysine which was higher in IV. Huang et al. (2006) reported similar essential amino acid AID than in IV, except the lysine AID being lower (0.76 vs 0.83). The AID of most essential amino acids were higher in II compared to Huang et al. (2006), except methionine and phenylalanine AID which were similar and tyrosine which was slightly lower compared to Huang et al. (2006). Bandegan et al. (2011) determined coefficient of variation (CV) from 0.6 to 3.9 % in individual amino acid contents between wheats, when crude protein varied from 14.8 to 18.4 %.

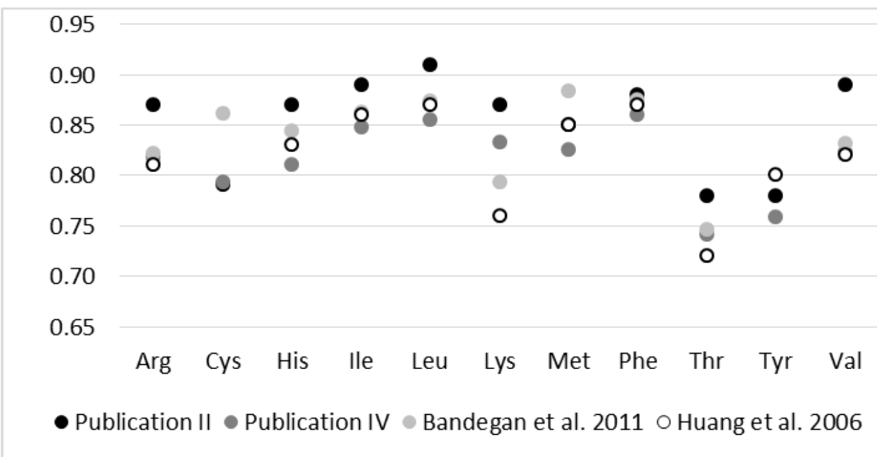

Figure 1. Wheat essential amino acid AID in II and IV in comparison to literature values.

The mean amino acid AID in dried barley diet in I was 8 %-units lower than in barely in II, the crude protein content of barleys being 125 and 132 g kg^{-1} DM in I and II, respectively. Bandegan et al. (2011) determined CV from 3.6 to 5.7 % in individual amino acid contents in barleys, crude protein content varying from 12.1 to 18.0% and noticed that the variation in amino acid content of barley samples was larger than in wheat samples. However, barley AID of amino acids in II were within the range determined by Bandegan et al. (2011), only AID of lysine and aspartic acid being slightly higher and methionine lower in II. In an previous experiment of Perttilä et al. (2001), the coefficient of barley lysine AID varied from 0.80 to 0.86 with barley volume-weight, which corresponds with the large variation in barley lysine AID.

Adedokun et al. (2009a) determined similar AID of most amino acids in maize, except that cysteine, glycine, tyrosine and threonine were lower and phenylalanine higher in II. In addition, Kong and Adeola (2013) determined a much lower AID of amino acids in maize, which they assumed to be attributed to the low diet amino acid and crude protein content.

Siegert et al. (2017) determined the effects of nitrogen fertilisation level and triticale genotype on the amino acid digestibility with caecetomised hens and found similar AID of amino acids in triticales, except AID of alanine, aspartic acid, histidine, isoleucine, leucine, lysine, methionine and valine were slightly higher in II, which may be the consequence of the determination method. According to Zuber et al. (2017), the amino acid digestibility in triticale is highly variable for hens and the variation could not be well explained by physical and chemical characteristics of the grain. However, they noticed that the fine structure of non-starch polysaccharides, the proportion and properties of various protein fractions and their effects on digesta viscosity, might be the reasons for variability. In addition, the proportion of the storage proteins and their digestibility varied between the 20 triticale genotypes they measured. Variation in the proportion of the storage proteins might be the reason for the variation in amino acid digestibility in other cereals, too.

Although dehulled oats contained less crude fibre than oats (45 vs 100 g kg^{-1} dry matter in II), their AID of amino acids were only 1–4 %-units higher compared to the oats; except the AID of cysteine which was much higher (0.38 vs 0.53; II).

Differences in the digestibility of nutrients as well as amino acids in the grains have previously been assumed to be caused by the differences in their nutrient composition, particularly by the fibre content and the differences in the contents of the anti-nutritive factors such as non-starch polysaccharides (Hughes and Choct, 1999). Mean amino acid AID and crude fibre content in the cereals is presented in Figure 2. The effect of the difference in the crude fibre content of the cereals on the mean amino acid AID is not clearly observed from Figure 2, except the higher fibre containing barley and oats having clearly lower amino acid AID than the other cereals.

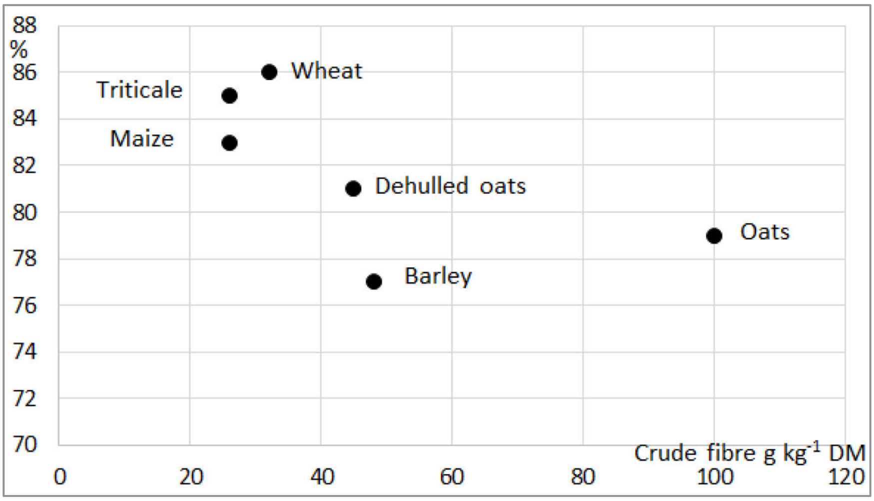

Figure 2. Mean apparent ileal amino acid digestibilities (%) and crude fibre content (g kg^{-1} dry matter) of the cereals (II).

Rodehutscord et al. (2016) presented that the mean concentration of the soluble β-glucans and arabinoxylans were 0.9, 2.0 and 24.1 g kg^{-1} DM and 12.6, 13.9 and 9.7 g kg^{-1} DM in triticale, wheat and barley, respectively. In II, concentrations of soluble β-glucans were higher, being 5.7, 6.0 and 35.7 g kg^{-1} DM in triticale, wheat and barley, respectively. According to Biel et al. (2014), dehulled oats contains 48 g and hulled oats 31 g total β-glucans kg^{-1} DM. In II, oats contained 40.3 and dehulled oats 49.2 g kg^{-1} DM total β-glucans, of which 39.8 and 43.4 g kg^{-1} DM were soluble in oats and dehulled oats, respectively. However, the non-starch polysaccharide content of maize is less than 1 g kg^{-1} (Choct 1997), but maize contains resistant starch, phytate and amylase inhibitors, which decreases the nutritional value of it (Cowieson 2005). β-Glucans and arabinoxylans decrease protein digestibility by impairing protein digestion, inhibiting amino acid absorption and increasing the secretion of the endogenous protein derived from the gut secretions and sloughed off epithelial cells to the digesta (Angkanaporn et al. 1994). In addition, digesta viscosity is increased (Choct and Annison 1992). Further, according to Rodehutscord et al. (2016), cereal grains of different genotypes differ in their chemical composition and variation in the soluble parts of β-glucans and arabinoxylans are high. Rodehutscord et al. (2016) indicated that the effects of growing location and agronomy on chemical composition of cereal grains still need to be investigated to obtain reliable values to be included in common feed tables and the impact of observed variations in carbohydrate factions and lignin on nutrient digestibility are not thoroughly evaluated. In addition, Zuber et al. (2017) noticed that the amino acid digestibility variation could be connected to the variable proportion and properties of various protein fractions that should not be overlooked.

In I, dried barley contained 45.5 g β-glucans per kg dry matter, but the negative effects of barley non-starch polysaccharides was avoided with enzyme supplementation and preservation. Air-tightly stored barley contained 44.9 and ensiled barley only 8.5 g β-glucans per kg dry matter. Ileal digesta viscosity decreased with β-glucanase supplementation from 12.9 centipoise to 9.6, from 15.0 to 6.0 and from 3.9 to 3.3 in dried, air-tightly stored and ensiled barley diet fed broilers (I). The AID of cysteine and proline on dried barley based diets were lower than in air-tightly stored and ensiled barley based diets without enzyme supplementation (I). The AID of alanine, glutamic acid, isoleucine, methionine, phenylalanine and proline were lower in air-tightly stored barley diets compared to that in ensiled barley diets. The β-glucanase enzyme improved the AID of amino acids in the dried barley containing diet for broilers, but had no effect on the air-tightly stored and on the ensiled barley containing diets for broilers (Figure 3), or on any diets for cockerels. Correspondingly, Wu et al. (2004) reported increased AID of protein with β-glucanase and Rutherfurd et al. (2007) noticed increased true ileal digestibility of amino acids with β-glucanase containing enzyme mixture supplementation. According to Dänicke et al. (1999), use of enzymes decreases mean retention time of digesta in the gizzard and large intestine and increases gut motility. Digesta viscosity and microbial fermentation are decreased and nutrient digestibility and rate of absorption are increased so that more feed can be consumed. However, β-glucanase increased only dry matter intake (g DM kg^{-1} $W^{0.75}$) in dried barley diet fed broilers compared to air-tightly preserved or ensiled barley diet fed broilers in I.

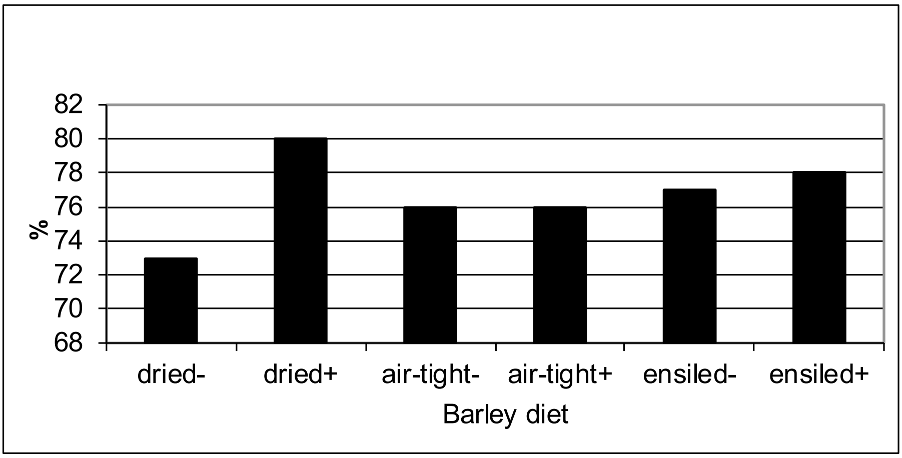

Figure 3. Mean amino acid AID (%) in dried, air-tightly preserved and ensiled barley diets fed with (+) or without (-) β-glucanase enzyme (I).

Table 5. Coefficients of apparent ileal digestibility of crude protein and amino acids (AA) in tested grains.

Ingredient	Barley	Wheat	Wheat	Oats	Triticale	Maize	Dehulled oats
Publication	II		IV	II			
Crude protein	0.74	0.85	0.82	0.77	0.83	0.81	0.79
Alanine	0.76	0.84	0.79	0.81	0.84	0.91	0.82
Arginine	0.80	0.87	0.82	0.91	0.87	0.88	0.90
Aspartic acid	0.76	0.82	0.77	0.82	0.83	0.84	0.83
Cystine	0.71	0.79	0.79	0.38	0.77	0.66	0.53
Glutamic acid	0.86	0.95	0.90	0.91	0.94	0.91	0.93
Glycine	0.72	0.82	0.77	0.66	0.80	0.75	0.69
Histidine	0.78	0.87	0.81	0.86	0.86	0.86	0.87
Isoleucine	0.80	0.89	0.85	0.88	0.88	0.85	0.89
Leucine	0.81	0.91	0.86	0.88	0.89	0.92	0.88
Lysine	0.81	0.87	0.83	0.86	0.89	0.84	0.87
Methionine	0.79	0.85	0.83	0.84	0.88	0.90	0.86
Phenylalanine	0.79	0.88	0.86	0.88	0.86	0.87	0.89
Proline	0.86	0.93	0.88	0.78	0.92	0.86	0.82
Serine	0.76	0.85	0.82	0.68	0.83	0.80	0.71
Threonine	0.71	0.78	0.74	0.72	0.78	0.73	0.75
Tyrosine	0.68	0.78	0.76	0.63	0.74	0.75	0.66
Valine	0.81	0.89	0.82	0.86	0.87	0.84	0.85
Mean AA	0.77	0.86	0.82	0.79	0.85	0.83	0.81

4.2.2. Protein ingredients

Among the protein ingredients, the crude protein AID was the highest in soybean cake, soybean meal and full-fat soybeans and decreased to rapeseed cake and further to rapeseed meal and meat and bone meal and finally being the lowest in full-fat rapeseeds (III) (Table 6). The AID of methionine was the lowest in full-fat rapeseeds and meat and bone meal, intermediate in full-fat soybeans and rapeseed meal and did not differ from rapeseed cake while being the highest in soybean cake, soybean meal and rapeseed cake. The AID of the other important amino acids, lysine, threonine and cysteine, were the highest in soybean cake and soybean meal and full-fat soybeans (III). The AID of lysine was intermediate in the rapeseed cake but did not differ from that in rapeseed meal and meat and bone meal. The AID of lysine was the lowest in full-fat rapeseeds but did not differ from meat and bone meal and rapeseed meal. The AID of cysteine was intermediate in full-fat rapeseeds and rapeseed cake and the lowest in rapeseed meal and meat and bone meal. The AID of threonine decreased from full-fat soybeans and rapeseed cake to rapeseed meal and meat and bone meal and further to full-fat rapeseeds. The AID of threonine in meat and bone meal did not differ from that in full-fat rapeseeds and rapeseed meal. The AID of amino acids in the soybean meal and in the rapeseed meal in IV differed greatly from those in III. However, the amino acids AID in the soybean meal in IV were higher than in rapeseed meal similarly as in III.

The variation in amino acid AID in soybean meal and rapeseed meal in literature was large (Figure 4 and 5) and not clearly connected to the chemical composition or especially the crude protein content of the products. However, despite some variations for individual amino acids, the AID of essential amino acids in soybean meal determined in the current assays (III and IV) support previous results of Adedokun et al. (2009a), Frikha et al. (2012) and Kong and Adeola (2013) (Figure 4). The essential amino acid AIDs in soybean meal were slightly higher in Huang et al. (2006) than in III and IV. In addition, Adedokun et al. (2009a) determined higher apparent digestibility of non-essential amino acids in soybean meal than in the current experiments. In De Coca-Sinova et al. (2008) AID of essential amino acids differed from other mentioned experiments; especially AID of cysteine being lower (Figure 4).

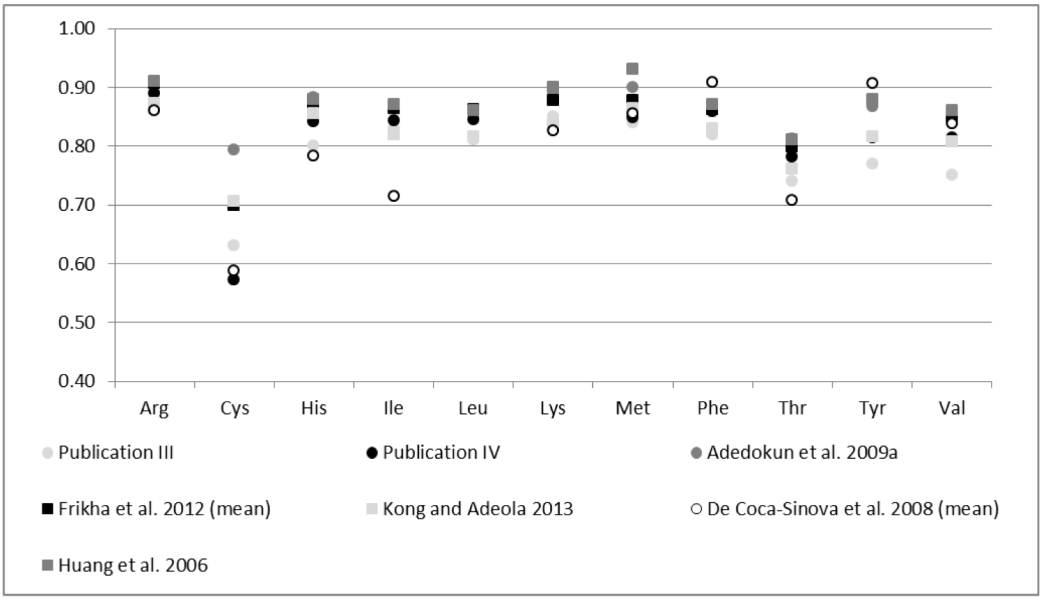

Figure 4. Soybean meal essential amino acid AID in III and IV in comparison to literature values.

Variation in AID of amino acids in rapeseed meal in literature was large (Figure 5). The essential amino acid AIDs of rapeseed meal were lower in III compared to most rapeseed meals tested in literature (Newkirk et al. 2003, Adedokun et al. 2009a, Toghyani et al. 2010, Kasparzak et al. 2017) (Figure 5). In Kasprzak et al. (2016), the average rapeseed meal crude protein content was much higher than in III and IV. In addition, the amino acid AIDs of the rapeseed meals were slightly lower in IV and much lower in III compared to Huang et al. (2006), although rapeseed meal crude protein content in Huang et al. (2006) was slightly lower than that in IV and much lower than that in III. However, the standard hexane extraction processed DK Cabernet rapeseed meal in Kasprzak et al. (2016) had almost identical amino acid AIDs than in IV. Gallardo et al. (2017) determined higher AID of lysine, threonine and aspartic acid and lower AID of cysteine with the difference method in rapeseed meal than in current experiments. However, the AID of other amino acids were between the values determined in III and IV. In addition, Tokhyani et al. (2015) and Newkirk et al. (2003b) determined values which were between the measured values in rapeseed meal in III and IV. Kasprzak et al. (2016) concluded that differences in rapeseed cultivation conditions such as environment and varieties, oilseed crushing and extraction process and especially heat-treatment influence the content of oil and protein and digestibility in the rapeseed meals and cakes, which might explain differences in digestibility results between previous and current experiments. The rapeseed meals in III and IV were from different batches and although probably similarly processed, the cultivation conditions and varieties of the raw materials are not known.

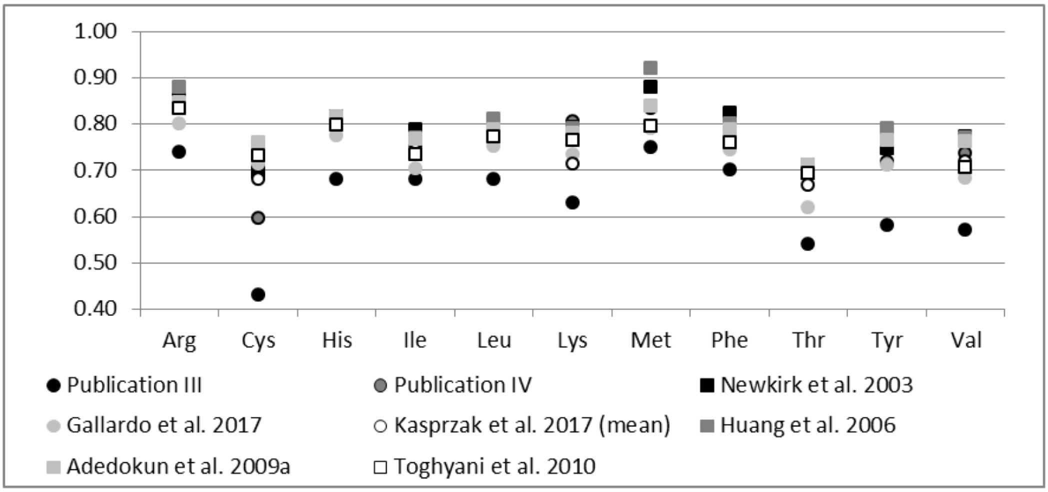

Figure 5. Rapeseed meal essential amino acid AID in III and IV in comparison to literature values.

There is limited amount of previous results of the amino acid AID of the full-fat soybeans and the full-fat rapeseeds in literature. However, Kaczmarek et al. (2019) reported 20 %-units higher crude protein precaecal digestibility in two samples of intact full-fat rapeseeds, which had crude protein contents of 201 and 210 g kg^{-1} DM, respectively, compared to III. In addition, the AID of amino acids in the soybean cake and in the rapeseed cake were also limited in literature. Kasprzak et al. (2016) found higher amino acid AID in most rapeseed cakes they determined than in the current experiments except for lysine AID, which was slightly higher in III. Woyengo et al. (2010) reported that rapeseed cake had slightly higher AID of amino acids than rapeseed meal, which corresponds to the current results in III.

In addition, full-fat rapeseeds had clearly the highest crude fibre content of the protein ingredients and it decreased from the rapeseed to the soybean products and to the meat and bone meal (III and IV). The crude fibre and lignin content of the rapeseed meal probably decreased its digestibility compared to the soybean meal (III and IV). The effect of crude fibre content on amino acid AID in protein ingredients is presented in Figure 6. In addition, Kasprzak et al. (2017) suspected that neutral detergent fibre (NDF) binds amino acids that are in the same fraction during processing rapeseed meals. Similarly, De Coca-Sinova et al. (2008) noticed, that the amino acid digestibility varied between soybeans and soybean products, and was negatively correlated with the NDF content of the product. Unfortunately, NDF content of the soybean and the rapeseed meals were not analysed in the current experiment. In addition, Frikha et al. (2012) noticed that the origin affects the crude protein content and in vitro nitrogen digestibility of soybean meals, performance results of broilers and coefficients of apparent and standardised ileal digestibility. The AID of cysteine was higher in the USA produced soybean meal than in that of other origins in Ravindran et al. (2014). Unfortunately, exact origin of the soybean meals used in the current experiments are not known.

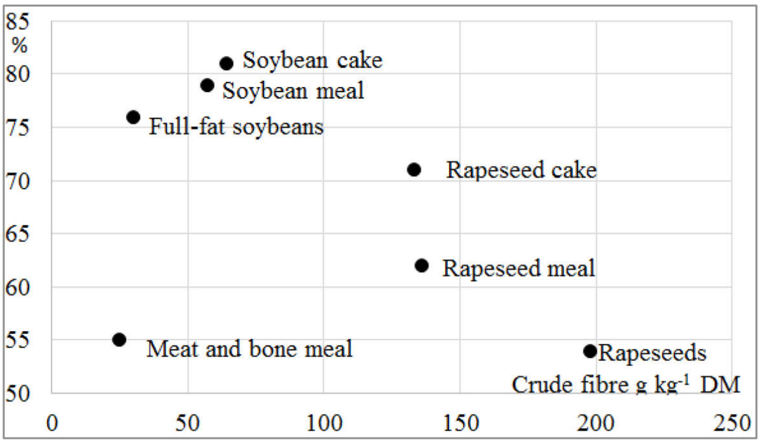

Figure 6. Mean apparent ileal amino acid digestibilities (%) and crude fibre content (g kg^{-1} dry matter) of the protein ingredients (III).

Adedokun et al. (2009a) determined much higher AID of most amino acids in meat and bone meal compared to the current experiment. Only the AID of histidine, aspartic acid and cysteine were higher in the current experiment. The quality and source of meat and bone meal is probably the reason for the difference in the determined digestibility values. Green and Kiener (1989) noticed that diets containing meat meal pass through the ileum at higher rate compared to soybean and sunflower meals due to higher concentrations of unsaturated fat and therefore, digestibility of amino acids are low in meat and bone meal. In addition, fat can affect diet palatability, thereby decreasing feed intake and amino acid digestibility, as was suspected in III.

According to Tahir and Pesti (2012), diet amino acid concentration had a positive effect on the amino acid AID and the effect is quantitatively similar for all amino acids except cysteine. Therefore, the concentration of amino acid in the experimental diet should be known to predict the digestibility of amino acids in ingredients. In addition, Green et al. (1987) have noticed that the intake of an amino acid affects its digestibility. In general, amino acid AID increases as the intake of the amino acid increases. The reason is that the level of the endogenous amino acids decreases relative to the undigested dietary amino acids according to increasing intake. As a conclusion, the higher the intake of an amino acid is the higher is the apparent digestibility of it. Contradictorily, at low feed intake the endogenous protein may significantly affect the apparent digestibility, which may explain the low digestibility values for the amino acids in meat and bone meal (IV) and large differences in amino acid AID values for protein ingredients in literature and in the current experiments.

Processing of the current protein ingredients is presented in III. All protein ingredients were heated to over 90˚C and the meat and bone meal and the full-fat soybeans to 130 ˚C. In the presented experiments, the contents of lysine and cysteine were lower in the heat-processed rapeseed meal compared to the non-processed full-fat rapeseeds (III), a finding that can be attributed to the heat-treatment during processing. According to Parsons (1996), amino acids most affected in processing appear to be lysine and cystine, since they can relinquish their last amino group to form a complex with carbohydrates, rendering them unavailable to the animal. In addition, the digestibility of cysteine in the soybean and rapeseed meals was remarkably low in IV. Newkirk et al. (2003a) and Newkirk et al. (2003b) concluded that processing of rapeseed meal samples decreased the content of lysine and digestibility of crude protein and lysine, and increased the variation in digestibility of lysine and other amino acids except of AID of glutamic acid, histidine, leucine and serine. The main reason for the variation of lysine digestibility was suspected to be the Maillard-reactions during processing. In addition, according to Toghyani et al. (2015), the processing of rapeseed products may increase solubility of fibre in them leading to decreased nutrient digestibility.

Cysteine content in trypsin and trypsin-inhibitors are high and therefore, binding of trypsin to inhibitors increases endogenous excretion of cysteine resulting in decreased AID of cysteine in products with high trypsin-inhibitor content (De Coca-Sinova et al. 2008). This might explain the large difference in the cysteine AID in the soybean meals in III and IV. In IV, the flow of cysteine was two-fold in the soybean meal, and 2.6-fold in rapeseed meal fed compared to the wheat fed broilers and 5.9- and 7.6-fold compared to the protein-free diet, respectively, resulting in the low AID of it. In addition, according to Frikha et al. (2012), processing affects greatly the activity and content of trypsin-inhibitors. However, the AID of cysteine in III correspond to results of Frikha et al. (2012) where the AID of cysteine ranged from 0.66 to 0.75 in different soybean meals, although the protein and the lysine contents were higher than in the current soybean meals varying from 518 to 564 g kg^{-1} dry matter and from 56.5 to 63.4 g kg^{-1} crude protein, respectively.

However, it is recommended nowadays that samples with low glucosinolate content would not be toasted because the non-toasted rapeseed meals contain higher levels of digestible amino acids than the conventional toasted rapeseed meals (Newkirk et al. 2003a). It might be possible that the soya and rapeseed products used in the current experiments are more processed than in other experiments in literature explaining the difference in chemical composition and digestibility compared to more recent experiments. In addition, soya and rapeseed cultivars have probably changed to some extent after the conduction of the currently presented experiments.

Table 6. Coefficients of apparent ileal digestibility of crude protein and amino acids (AA) in tested protein ingredients.

Ingredient	Meat and bone meal	Full-Fat soya beans	Soya-bean cake	Soya-bean meal	Soya bean meal	Rape-seeds	Rape-seed cake	Rape-seed meal	Rape-seed meal
Publication	III				IV	III			IV
Crude protein	0.58	0.75	0.80	0.78	0.82	0.48	0.67	0.58	0.73
Alanine	0.60	0.81	0.86	0.85	0.83	0.59	0.68	0.63	0.79
Arginine	0.69	0.77	0.83	0.80	0.89	0.57	0.74	0.66	0.85
Aspartic acid	0.66	0.75	0.86	0.84	0.79	0.59	0.81	0.75	0.72
Cysteine	0.65	0.78	0.85	0.82	0.57	0.58	0.78	0.70	0.60
Glutamic acid	0.63	0.76	0.80	0.77	0.83	0.59	0.62	0.51	0.84
Glycine	0.50	0.74	0.80	0.78	0.78	0.39	0.65	0.57	0.72
Histidine	0.72	0.83	0.89	0.86	0.84	0.68	0.83	0.74	0.80
Isoleucine	0.29	0.77	0.77	0.76	0.84	0.52	0.65	0.57	0.75
Leucine	0.34	0.67	0.62	0.63	0.85	0.53	0.53	0.43	0.79
Lysine	0.57	0.82	0.82	0.81	0.88	0.68	0.79	0.74	0.81
Methionine	0.66	0.74	0.76	0.73	0.85	0.56	0.66	0.56	0.84
Phenylalanine	0.51	0.78	0.82	0.80	0.86	0.64	0.75	0.68	0.79
Proline	0.48	0.68	0.76	0.74	0.80	0.43	0.64	0.54	0.71
Serine	0.40	0.73	0.78	0.77	0.82	0.28	0.66	0.58	0.71
Threonine	0.58	0.79	0.84	0.83	0.78	0.50	0.73	0.68	0.70
Tyrosine	0.52	0.69	0.80	0.75	0.81	0.49	0.70	0.57	0.72
Valine	0.56	0.75	0.84	0.81	0.82	0.53	0.77	0.68	0.74
Mean AA	0.55	0.76	0.81	0.79	0.81	0.54	0.71	0.62	0.76

4.3. Methods measuring amino acid digestibility

In current experiments, the amino acid digestibility was measured from ileum as apparent digestibility with direct (II, III, IV) and difference method (I). In general, there seems to be large variation in the amino acid digestibility results obtained depending on the methods used. Previously the amino acid digestibilities were measured as total tract digestibility and excreta was collected from force-fed intact (Sibbald 1979) or caecectomised birds (Parsons 1996, Green et al. 1987, Gruhn et al. 1989). The effect of the digestion and fermentation reactions in the lower digestive tract on the amount of available amino acids is known to be negligible, because little or no amino acids are absorbed after the ileum. However, diet carbohydrates influences bacterial activity in hindgut and change the microbial population, fermentation products and the amino acid content of excreta in the lower gastro-intestinal tract (Ravindran et al. 1999).

In current experiments, the ileal digestibility method was used, where the digesta is collected from the distal part of the ileum and the problems as a result of the bacterial fermentation in hindgut are avoided. In the ileal digestibility method, the digesta can be collected through an intestinal cannula or by a slaughter method. Fistulated birds can be used for several tests, but obtaining sufficient amounts of the digesta is laborious and the insertion of the fistula requires considerable surgical skills (Lemme et al. 2004). Therefore, slaughter method is used commonly nowadays and was used in current experiments. With the slaughter method, birds are killed humanely, the small intestine is immediately surgically exposed and the digesta is collected from the distal part of the ileum. Several birds are used to get sufficient quantities of digesta for analysis (Lemme et al. 2004).

In addition, the region of ileum for sampling (half to two thirds and to the total ileum) has varied in previous experiments (Adedokun et al. 2007b, Kong and Adeola, 2013 and Ravindran et al. 2004). However, according to Poureslami et al. (2012), the digestive section (terminal or entire ileum) of collection of digesta, did not affect the AID of amino acids in broilers. The method of ileal digesta collection in current experiments (squeezing, not flushing) may have released epithelial cells, mucus and mucin from the intestinal wall to the digesta increasing the amount and concentration of mucosal and mucus amino acids. According to Poureslami et al. (2012), flushing procedure to collect digesta for digestibility determination is a more accurate method than squeezing the digesta from ileum. In addition, Adedokun et al. (2011) concluded that digesta should not be squeezed but flushed with distilled water to minimize damage to the epithelial surface of the ileum.

Furthermore, euthanizing method of birds has been different in previous experiments, although it has not been noticed to affect composition of digesta of birds (Summers and Robblee 1985). Adeola and Kong (2013) and Adedokun et al. (2007b) used carbon dioxide asphyxiation for euthanizing, Ravindran et al. (2004) used intracardial injection of diluted sodium bentabarbitone solution and Golian et al. (2008) euthanized birds by cervical dislocation.

The amino acid AIDs in I were significantly lower in the broilers than in the cockerels (Figure 7). The barley preservation method did not affect the amino acid AID with cockerels. Interactions between the animals and the barley preservation were significant for the AID of arginine, aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, proline, serine and threonine. Furthermore, AME_n values for the adult cockerels were higher than for the growing broilers. Age and species originating differences in the amino acid digestibility values in different ingredients are reported widely in literature (Adedokun et al. 2007b and 2014, Huang et al. 2006, Toghyani et al. 2015, Szczurek 2009, 2010) reflecting the higher amino acid digestibilities with cockerels compared to broilers in I. Observed differences are explained by the differences in the stage of development of the digestive tract and the amount of feed intake. Digesta transit time changes with age and young broilers have immature digestive tracts and lower endogenous enzyme activities compared to adult cockerels. Therefore, AME values increases as the ability of broilers to digest feed increases (Scott et al. 1998). According to Toghyani et al. (2015), mucin turnover decreases according to broiler age and therefore AID of glutamic acid, aspartic acid, serine and threonine decrease. In addition, the anti-nutritive effects of the non-starch polysaccharides such as β-glucans are more pronounced in younger animals (Pettersson et al. 1991) corresponding to the positive effect of β-glucanase supplementation in I. Unfortunately, ileal digesta viscosity was not measured form the cockerels because of small ileal digesta sample size in I and therefore the digesta viscosity values of broilers and cockerels cannot be compared. However, the feed intakes of the cockerels were lower than those of the broilers. The cockerels were fed at maintenance level compared to broilers getting feed *ad libitum*. The feed intake merely can have an influence on the AID of nutrients due to variations in the amount of the exogenous material in total digesta and the rate of digesta passage (Kadim and Moughan 1997).

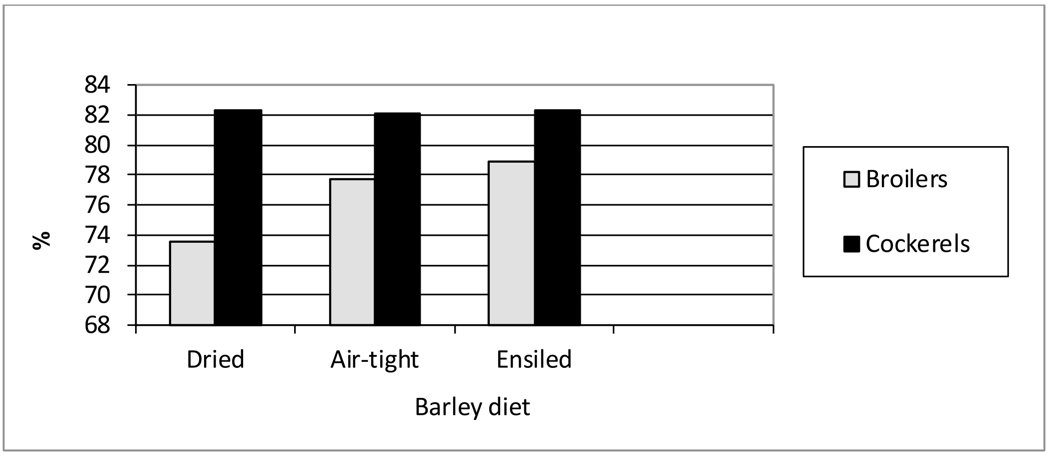

Figure 7. Mean amino acid AID in dried, air-tightly preserved and ensiled barley diets for broilers and cockerels (I).

Amino acid AID was measured with difference method in experiment I and with direct method in other digestibility experiments (II, III and IV). In general, the digestibility coefficients are assumed to be additive in difference method. It is assumed that there is no interaction between the basal diet and the test ingredient. The difference method is criticized because it may under- or over-estimate the AID of amino acids because of the differences in intake from the basal and the test ingredients. In I, the AID of amino acids in the basal diets tended to be lower compared to the other diets resulting in a large variation and over-estimation of the calculated barley digestibility values. The digestibility of nutrients in the basal diet may have decreased after incorporation with the barleys because of the content of anti-nutritional β-glucans of the barleys as in Kadim et al. (2002). However, the effect of diet composition should be as normal as possible also in the direct method, because the composition of the test diet affects the digestive processes. The direct method is not always possible to use, because of abnormal physiological state when diet is not nutritionally balanced with tested ingredients such as cereals. The diet composition has a direct effect on the endogenous secretions (Lemme et al. 2004) and abnormal diet composition may therefore lead to erroneous AID values. The direct method is also criticized, because the calculation of the digestibility values assumes that the amino acid digestibility of the diet is representative of that of the feed ingredient and the possible interactions of the ingredients is forgotten.

4.4. Basal endogenous amino acid flow

Endogenous amino acids are the amino acids that do not originate from the diet. Digestive secretions such as bile, saliva, gastric and pancreatic secretions, enzymes, mucoproteins, serum albumin and amides, sloughed intestinal epithelial cells and bacterial protein are excreted to the digesta and the amino acids they contain misrepresent the actual digestibility values of dietary ingredients (Adedokun et al. 2011). The basal losses are related to the dry matter intake and are independent of the raw material or the diet composition. The specific losses are influenced by the composition of the raw material, such as the presence of anti-nutritional factors and protein content that may stimulate endogenous secretions (Adedokun et al. 2011). Basal endogenous losses can be measured with a protein-free diet, feeding casein or wheat gluten and with a regression method. Determination of specific losses is possible with an isotope-dilution and a homoarginine technique (Lemme et al. 2004). In IV, the composition of the endogenous secretion (Table 7) was measured with the protein-free diet to calculate standardised ileal digestibility values (SID) for wheat, soybean meal and rapeseed meal for broilers (Table 8). According Adedokun et al. (2009b), the protein-free diet method is suitable to measure the endogenous secretion, because it provides the lowest correction and consistent values across laboratories and it does not result in over 100 % SID amino acid values for the low protein ingredients such as grains.

In IV, the predominant endogenous amino acids in digesta from the distal ileum of the protein-free diet fed to broilers were aspartic acid and glutamic acid and the lowest ones present were methionine and histidine similarly as in Ravindran et al. (2004), Adedokun et al. (2007b), Golian et al. (2008) and Kong and Adeola (2013) (Table 7), although the concentration of most amino acids were lower than in Ravindran et al. (2004) and Kong and Adeola (2013) and higher than in Adedokun et al. (2007b) and Golian et al. (2008). Ravindran et al. (2004), Golian et al. (2008) and Kong and Adeola (2013) found that also the flow of tryptophan was one of the lowest, but it was not analysed in the current experiment.

Table 7. Basal endogenous ileal amino acid flows (mg kg^{-1} dry matter intake) in broilers fed protein-free diet.

Amino acid	IV	Kong and Adeola 2013	Golian et al. 2008	Adedokun et al. 2007b	Ravindran et al. 2004
Alanine	241	377	217	177	293
Arginine	237	369	203	168	280
Aspartic acid	554	752	430	340	607
Cysteine	216	218	143	136	262
Glutamic acid	541	915	492	420	721
Glycine	319	431	245	205	508
Histidine	131	160	91	73	158
Isoleucine	188	375	200	162	287
Leucine	282	564	298	251	439
Lysine	196	382	173	181	209
Methionine	108	100	65	50	101
Phenylalanine	196	345	420	154	287
Proline	360	519	289	240	nd
Serine	341	505	343	260	424
Threonine	368	582	434	274	512
Tryptophan	nd	87	71	nd	95
Tyrosine	397	287	nd	124	253
Valine	365	531	270	214	417

In general, mucus glycoprotein contains much threonine and mucin glutamic acid, aspartic acid, serine and glysine (Huang et al. 2006). According to Lien et al. (1997), threonine, serine, proline and valine contents are also high in pig mucin. Adeola et al. (2016) reviewed nine studies reporting that predominant endogenous amino acids are glutamic acid, aspartic acid, threonine, serine, leucine, valine and proline, and the lowest ones tryptophan and methionine when protein-free diet method was used. In IV, the basal ileal endogenous amino acid flow decreased in the order from tyrosine to threonine and further to valine, proline, serine and glysine.

The reason for variation in basal ileal endogenous flows of amino acids might be that gut mucin is degraded and renewed non-regularly and it is influenced by the nature of the diet and starvation (Adedokun et al. 2011). In IV, 24-h fasting and short time feed-serving before collection of the ileal digesta samples may have affected the amount and composition of endogenous secretion. Kadim and Moughan (1997) preferred a 24-h fasting period, because between animal variation for measured apparent ileal nitrogen digestibility was lower after a 24-h fasting period than after a 12-h fasting or when fed continuously.

Furthermore, the observed discrepancy in amounts of endogenous secretions may be partly explained by the measuring method. In addition, Adedokun et al. (2007a) reported that the age and species of birds influence the endogenous amino acid flow, which complicates comparisons of results with previous experiments.

The type of fibre changes the viscosity and rate of digesta passage and mucin excretion and therefore, cellulose is added to protein-free diets to maintain the normal water absorption, digesta viscosity and mucin secretion (Adeola et al. 2016). Adedokun et al. (2011) reviewed several articles and concluded that the level and nature of fibre may explain some of the inconsistencies in endogenous flows in different studies. In addition, Adedokun et al. (2009b) determined lower endogenous amino acid secretion with 21-d old broilers fed a protein-free diet containing 50 g kg^{-1} Solkafloc compared to 116 g kg^{-1} cellulose in current experiment. However, endogenous flow of arginine, histidine, methionine, alanine, aspartic acid, glycine, proline and serine in IV were close to the values determined in laying hens by Adedokun et al. (2009b), except that the flow of cysteine being slightly higher.

Furthermore, Adedokun et al. (2011) noticed that electrolytic balance and relation of starch and dextrose in protein-free diet influenced the composition and amount of endogenous secretion. In IV, the ratio of glucose to starch was 1:1 leading to a higher endogenous amino acid secretion than observed by Adedokun et al. (2007b), who determined endogenous amino acid flow for 15-d and 21-d old broilers feeding a protein-free diet containing a 3.8 fold amount of dextrose related to corn starch. In addition, replacing maize starch by dextrose, the oil content of the diet and the type of used marker has an effect on endogenous amino acid secretion according to the review of Adeola et al. (2011).

Using a protein-free diet can be criticized because the endogenous amino acid flow is determined in a physiologically abnormal state. According to Ravindran et al. (2004), Adedokun et al. (2007b) and Golian et al. (2008), the proportion of endogenous secretion is dependent on and increases by the level of protein in the diet. Dietary protein content may be the most important reason to the differences in amounts of endogenous secretion of amino acids measured with other methods than a protein-free diet. The reason is the increased enzyme secretion and/or increased mucus hydrolysis leading to increased mucin concentration in the digesta (Adedokun et al. 2011). However, according to Golian et al. (2008) for the majority of amino acids, the estimates of basal ileal endogenous flow, determined with either the protein-free diet or the regression method involving graded levels of casein or enzyme hydrolysed casein diets, were comparable. Therefore, using the protein-free diet for measuring endogenous secretion allows comparing feed ingredients more accurately, leading to a greater consistency in feed evaluation.

4.5. Standardised ileal amino acid digestibility

In IV, the mean amino acid SID was numerically the highest in soybean meal, but did not differ significantly from that in wheat (Table 8.). The lowest mean amino acid SID was in rapeseed meal. The SID of arginine and histidine were similar in rapeseed and wheat and SID of cysteine and glutamic acid in rapeseed and soybean meal. However, amino acids SID in wheat, soybean and rapeseed meal were in agreement with Hoehler et al. (2006), except that SID of cysteine in soybean and rapeseed meal were much lower.

Table 8. Apparent (AID) and standardised (SID) ileal digestibility of amino acids in soybean meal, rapeseed meal and wheat measured with broilers in experiment IV.

Ingredient	Soybean meal		Rapeseed meal		Wheat	
	AID	SID	AID	SID	AID	SID
Mean	0.81	0.85	0.76	0.79	0.82	0.87
Alanine	0.83	0.86	0.79	0.81	0.79	0.85
Arginine	0.89	0.91	0.85	0.87	0.82	0.86
Aspartic acid	0.79	0.81	0.72	0.76	0.77	0.86
Cysteine	0.57	0.65	0.60	0.65	0.79	0.86
Glutamic acid	0.83	0.85	0.84	0.86	0.90	0.92
Glycine	0.78	0.81	0.72	0.75	0.77	0.83
Histidine	0.84	0.87	0.80	0.83	0.81	0.85
Isoleucine	0.84	0.86	0.75	0.78	0.85	0.89
Leucine	0.85	0.86	0.79	0.81	0.86	0.89
Lysine	0.88	0.90	0.81	0.82	0.83	0.89
Methionine	0.85	0.89	0.84	0.86	0.83	0.88
Phenylalanine	0.86	0.88	0.79	0.82	0.86	0.89
Proline	0.80	0.84	0.71	0.74	0.88	0.91
Serine	0.82	0.85	0.71	0.75	0.82	0.88
Threonine	0.78	0.83	0.70	0.74	0.74	0.84
Tyrosine	0.81	0.87	0.72	0.78	0.76	0.86
Valine	0.82	0.85	0.74	0.77	0.82	0.87

Wheat amino acids SID were similar than in Hoehler et al. (2006), except cysteine, histidine, isoleucine were higher, methionine and threonine slightly higher and lysine slightly lower compared to IV (Figure 8). In addition, wheat amino acid SID were similar than in Bandegan et al. (2011), SID of histidine, methionine being slightly lower, SID of phenylalanine, cysteine and glutamic acid being slightly lower and lysine higher in IV. In contrary, Osho et al. (2019) reported much lower amino acid SID in wheat compared to IV. In addition, Szczurek (2009) determined lower SID of amino acids in wheat, except a higher histidine SID than in IV.

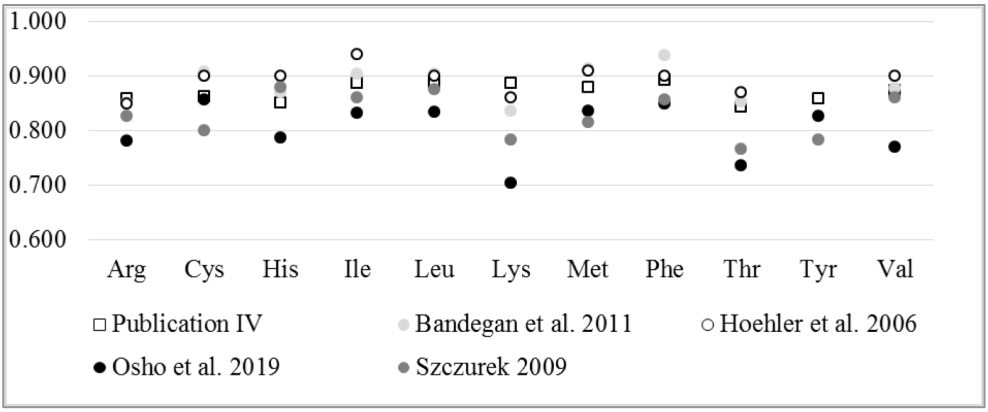

Figure 8. Wheat amino acid standardized ileal digestibilities in IV in comparison to literature values.

Kong and Adeola (2013) reported ±0-5 %-units difference in amino acid SID values in soybean meal compared to current results (IV) (Figure 9). In addition, SID of cysteine was much lower in IV. The SID of essential amino acids in soybean meal were much lower in Ravindran et al. (2017). The SID of essential amino acids in soybean meal in IV were rather similar than in Adedokun et al. (2009a). However, SID of almost all non-essential amino acids in soybean meal were higher in Adedokun et al. (2009a) than in IV. In addition Frikha et al. (2012) determined higher amino acid SID in soybean meal than in IV. Szczurek (2009) measured higher SID of cysteine and glycine in soybean meal while the other amino acids SID were similar than in IV.

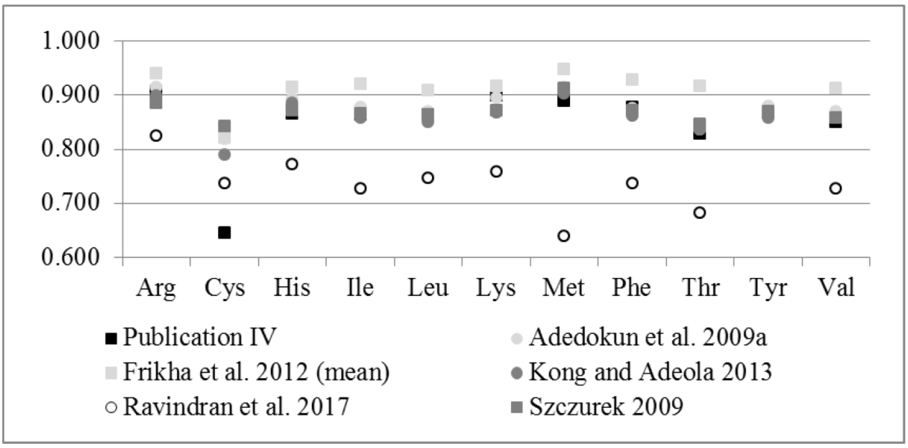

Figure 9. Soybean meal amino acid standardized ileal digestibilities in IV in comparison to literature values.

The SID of lysine was 3 %-units higher and proline lower, all other amino acids SID being similar in rapeseed meal in IV compared to Adedokun et al. (2009a) (Figure 10). In addition, SID of most amino acids in rapeseed meal corresponded well to Gallardo et al. (2017). However, SID of lysine in rapeseed meal was higher and SID of methionine, cysteine and proline were lower in IV. Osho et al. (2019) reported similar amino acid SID in rapeseed meal, except histidine, lysine, threonine, valine and serine being lower and cysteine higher than in IV. In Kasprzak et al. (2017), SID of isoleucine and cysteine in rapeseed meal were higher and methionine lower than in IV. All other amino acid SID corresponded well with current results (IV). Szczurek (2009) determined higher SID of methionine, cysteine and tyrosine in rapeseed meal than in IV. Other amino acid SID in rapeseed meal corresponded well with IV. In Toghyani et al. (2015), SID of histidine, isoleucine, leucine, threonine, valine, cysteine, glycine, proline and serine were higher in rapeseed meal for 24 day old broilers than in IV. The SID of cysteine, glycine and proline in rapeseed meal were also higher for 10 day old broilers than in IV. Arginine, leucine, lysine, phenylalanine and tyrosine SID were higher in rapeseed meal compared to solvent-extracted rapeseed meal in Woyengo et al. (2010).

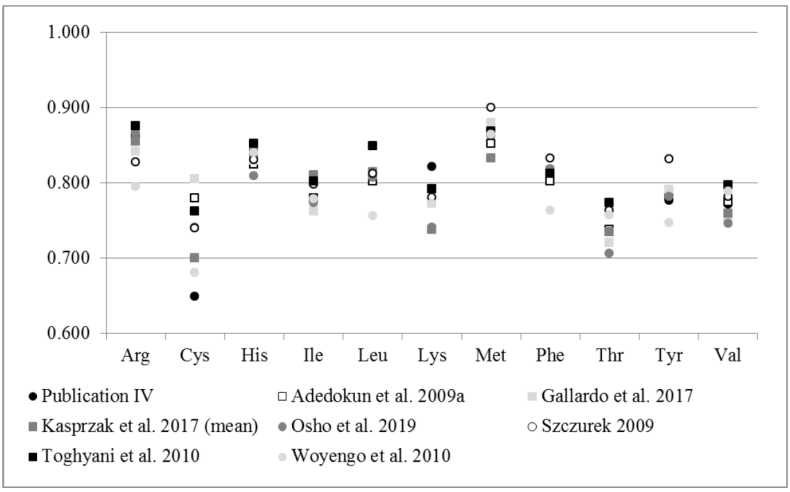

Figure 10. Rapeseed amino acid standardized ileal digestibilities in IV in comparison to literature values.

The SID of amino acids were 3.2, 3.1 and 5.4 %-units higher than amino acid AID in soybean meal, rapeseed meal and wheat in IV, respectively (Table 8). The difference between determined amino acid AID and SID varied from 1 to 8 %-units in soybean meal, from 1 to 6 %-units in rapeseed meal and from 2 to 10 %-units in wheat for individual amino acids. The AID of lysine was slightly lower (1-2 %-units) than the SID of lysine in soybean meal and rapeseed meal. Wheat lysine AID was 6 %-units lower than lysine SID. Highest difference was observed for threonine and tyrosine apparent and standardised digestibility values in wheat (10 %-units).

In Toghyani et al. (2015), the difference between AID and SID was 3.5 and 2.5 %-units for rapeseed meal in 10 and 24 day old broilers corresponding to current results. Similarly to IV, Toghyani et al. (2015) determined highest difference in threonine but also phenylalanine, aspartic acid and serine differed in AID and SID in rapeseed meal. Lemme et al. (2004) noticed that the difference between standardised and apparent ileal digestible amino acids ranged between 0 and 17 percentage units for cereal grains but only between 0 and 7 percentage units for plant protein sources and animal by-products. In addition, Hoehler et al. (2006) reviewed AID and SID of amino acids in different ingredients and noticed that the largest difference from AID to SID was seen in grains. Furthermore, they determined that the digestibility of threonine was most affected by the standardisation and noticed that it is probably related to the high content of threonine in endogenous proteins. Kong and Adeola (2013) and Adedokun et al. (2008) noticed that the effect of higher endogenous losses on difference between AID and SID would be more pronounced at lower amino acid levels in the diet such as in maize compared to soybean meal.

Diet fibre content changes the viscosity and passage rate of ileal digesta influencing the secretion of mucin and epithelial cell turnover. In addition, anti-nutritive factors and feed intake effect on endogenous secretions and diet protein have a dose-dependent effect on specific ileal endogenous amino acid flows (Bandegan et al. 2011). Using protein-free diet, only the basal amino acid endogenous secretion can be measured and the effects dependent on the tested ingredients remain on SID values after basal endogenous excretion is subtracted from apparent values. Hence the difference between AID and SID digestibilities of raw materials with low amino acid levels and high fibre content, such as cereals and grain legumes, are most affected (Figure 11).

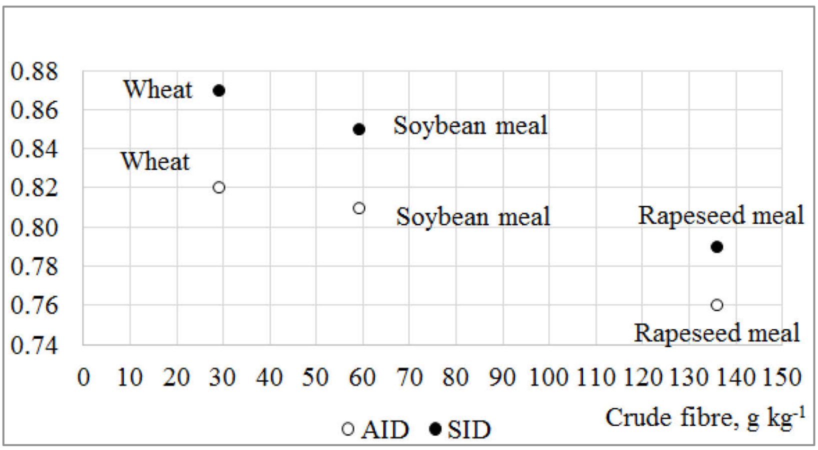

Figure 11. Amino acid apparent (AID) and standardized (SID) ileal digestibilities and crude fibre content of wheat, soybean meal and rapeseed meal in IV.

4.6. Feed formulation

The results from III imply that formulating diets based on ileal digestible amino acids allows to include more poorly digestible protein sources into the diets and thereby to lower the feed costs. Ileal digestible lysine based feed formulation has been noticed to be superior in other experiments (Dalibard and Pailard, 1995, Douglas and Parsons, 1999). Bryden and Li (2010) reported that the inclusion levels of poor quality protein sources can be increased when diets are based on apparent ileal amino acid digestibility values and observed depressions in broiler performance optimised according to total amino acid basis can be overcome. Previously, the difference between AID and AED of amino acids has been noticed to vary depending on the dietary ingredient and the amino acid in question (Ravindran et al. 1999, Kadim et al. 2002). In the presence of poorly digestible carbohydrates (example in grains), the microbial synthesis increases endogenous secretion and amount of nitrogen in lower digestive tract leading to lower AED than AID values of the amino acids (Ravindran et al. 1999). Microbial synthesis and endogenous secretion can also increase when the protein ingredients with poorly digestible protein (for example meat and bone meal) are fed leading to an increase in difference between amino acid AID and SID values. Therefore, the feeding values of poorly digestible protein sources increases, when the ileal digestible values, and furthermore, the standardised values, compared to the apparent values are used (Dalibard and Pailard 1995, Douglas and Parsons 1999, Bryden and Li 2010 and Hoehler et al. 2006). The difference between amino acid AID or SID and AED is smaller in highly digestible ingredients such as soybean meal and maybe therefore, ileal digestible amino acids based feed formulating system has not yet become widely used. The need for more precise and economic feeding increases the advantages of amino acid SID based feed formulation in future.

Digestibility values of individual ingredients should be additive when combined in diet formulations. However, there might be associative effects when high levels of poorly digestible ingredients are used. Individual ingredients may have specific effects on endogenous secretions. In addition, Kong and Adeola (2013) noticed that the measured AID for most amino acids in mixed feed diets were higher than the values predicted from the AID for an individual ingredient and the lack of additivity might be derived from underestimation of digestibility for low-protein ingredients when apparent values are used. If the diet contains feed ingredients, which increase endogenous enzyme production, digestibility of other ingredients may improve. On the contrary, feed ingredients containing anti-nutritive factors and high fibre content may decrease digestibility of other ingredients in the diet. In addition, in III, the high inclusion rate of rapeseed meal may account for the lower performance responses to the digestible lysine for the diets containing the rapeseed because of its high fibre content.

The advantages of SID values are that they are independent of basal endogenous secretion and are therefore more additive in diet formulation than the apparent ones. In addition, the SID values are independent of diet protein level. Unfortunately, comparing the standard and apparent ileal amino acid digestibility values in diet formulation for broiler performance experiments are scarce. Hoehler et al. (2006) formulated experimental diets based on total amino acids or standardised amino acids containing corn or sorghum and two inclusion levels of cottonseed meal and showed that using SID values resulted in an improved performance compared to total amino acids in feed formulation. In addition, they concluded that the higher the inclusion of low digestibility raw materials, the more important is to formulate on standardised digestible amino acid basis to avoid reduction in performance results.

5. Conclusions

1. The AID of individual amino acids varied between cereals. The AID of most amino acids were highest in wheat, triticale and maize, intermediate in dehulled oats and lowest in oats and barley.
2. Soybean products have higher AID of amino acids than rapeseed products. Meat and bone meal and full-fat soybeans and rapeseeds had clearly the lowest amino acid digestibilities.
3. Preservation method affected the AID of amino acids in barley. The AID of cysteine and proline in dried barley containing diets were lower than in air-tightly stored and ensiled barley containing diets. The AID of alanine, glutamic acid, isoleucine, methionine, phenylalanine, and proline were lower in air-tightly stored barley compared to that in ensiled barley.
4. Amino acids AID in dried, air-tightly stored and ensiled barley diets were lower in broilers than in cockerels. The β-glucanase enzyme improved the AID of amino acids in the dried barley containing diet for broilers, but had no effect on the air-tight stored and on the ensiled barley containing diets for broilers, or on any diets for cockerels.
5. Differences in amino acid digestibility values of tested ingredients were explained by their analysed chemical composition. Ingredients with higher fibre content such as cereals and rapeseed products had lower amino acid digestibilities.
6. The predominant endogenous amino acids in digesta from the distal ileum of broilers fed a protein-free diet (basal endogenous secretion) were aspartic acid and glutamic acid and the lowest ones present were methionine and histidine.
7. Basal endogenous amino acid flow was used to calculate standardised amino acid digestibilities for ingredients. The standardised amino acid digestibility values were highest in soybean meal, intermediate in wheat and lowest in rapeseed meal.
8. The difference between amino acid AID and SID was smaller in soybean meal and rapeseed meal compared to wheat.
9. The results from performance experiment implied that formulating diets, which contain rapeseed meal and meat and bone meal, based on ileal digestible amino acids allows better broiler performance compared to total amino acid based feed formulation. Variation in the difference between amino acid AID and SID values of different ingredients explains the need to standardise and use standardised amino acid SID values in feed formulation.
10. The amino acid digestibility values measured in current experiments could be added to Finnish feed tables. This would allow formulate more accurately the crude protein-feeding of broilers fed diets composed of different feed ingredients.

6. Future perspectives

1. Poultry researches have to harmonize methods to measure amino acid digestibilities and endogenous secretion. The variability in methods measuring amino acid digestibility and endogenous secretions is still great and confusing. The methods should be standardized before reliable values for feed formulation can be presented. In current experiments, the use of protein-free diet gave reliable results to compare SID of amino acids of different types of feed ingredients.
2. Requirements of broilers for ileal digestible amino acids should be more clearly defined and scientifically published.
3. More experiments, where diet formulating based on standardised, apparent or total amino acids are compared, should be performed to get adequate evidence to select the most appropriate way to determine protein value of ingredients and formulate diets for growing broilers.
4. The effect of different protein and fibre fractions and changes in their contents on amino acid digestibility in feed ingredients produced under different agronomic and environmental conditions and derived from different genotypes of plants should be clarified more precisely.
5. More information of ingredient processing and storage on amino acid digestibility is needed.

References

Adedokun, S.A., Adeola, O., Parsons, C.M., Lilburn, M.S. and Applegate, T.J. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poultry Science 87: 2535-2548.

Adedokun, S.A., Adeola, O., Parsons, C.M., Lilburn, M.S. and Applegate, T.J. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poultry Science 90: 1737-1748.

Adedokun, S.A., Jaynes, P., Abd El-Hack, M.E., Payne, R.L. and Applegate, T.J. 2014. Standardized ileal amino acid digestibility of meat and bone meal and soybean meal in laying hens and broilers. Poultry Science 93: 420-428.

Adedokun, S.A., Parsons, C.M., Lilburn, M.S., Adeola, O. and Applegate, T.J. 2007a. Comparison of ileal endogenous amino acid flows in broiler chicks and turkey poults. Poultry Science 86: 1682-1689.

Adedokun, S.A., Parsons, C.M., Lilburn, M.S., Adeola, O. and Applegate, T.J. 2007b. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poultry Science 86: 2590-2597.

Adedokun, S.A., Utterback, P., Parsons, C.M., Adeola, O., Lilburn, M.S. and Applegate, T.J. 2009a. Comparison of amino acid digestibility of feed ingredients in broilers, laying hens and caceotomised roosters. British Poultry Science 50: 350-358.

Adedokun, S.A., Utterback, P., Parsons, C.M., Adeola, O., Lilburn, M.S. and Applegate, T.J. 2009b. Comparison of endogenous amino acid flow in broilers, laying hens and caceotomised roosters. British Poultry Science 50: 359-365.

Adeola, O. and Kong, C., 2013 Additivity of amino acid digestibilty in corn and soybean meal for broiler chickens and White Pekin ducks. Poultry Science 92: 2381-2388.

Adeola, O., Xue, P.C., Cowieson, A.J. and Ajuwon, K.M., 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Animal Feed Science and Technology 221: 274-283.

Åman, P., Graham, H. & Tilly, A.-C., 1989. Content and solubility of mixed-linked (1□3), (1□4)-β-D-glucan in barley and oats during kernel development and storage. Journal of Cereal Science 10: 45-50.

Angkanaporn, K., Choct, M., Bryden, W. L., Annison, E. F., Annison, G., 1994. Effects of wheat pentosans on endogenous amino acid losses in chickens. Journal of the Science of Food and Agriculture 66: 399-404.

Bandegan, A., Golian, A., Kiarie, E., Payne, R.L., Crow, G.H., Guenter, W. and Nyachoti, C.M., 2011. Standardized ileal amino acid digestibility in wheat, barley, pea and flaxseed for broiler chickens. Canadian Journal of Animal Science 91: 103-111.

Biel, W., Jacyno, E. and Kawęcka, M., 2014. Chemical composition of hulled, dehulled and naked oat grains. South African Journal of Animal Science 44: 189-197.

Bryden, W.L. and Li, X., 2010. Amino acid digestibility and poultry feed formulation: expression, limitations and application. Revista Brasileira de Zootecnia 39: 279-287.

Cave, N.A., 1988. Bioavailability of amino acids in plant feedstuffs determined by in vitro digestion, chick growth assay, and true amino acid availability methods. Poultry Science 67: 78-87.

Choct, M., 1997. Feed non-starch polysaccharides: chemical structures and nutritional significance. Feed Milling Int., 13-16.

Choct, M. and Annison, G., 1992. Anti-nutritive effect of wheat pentosans in broiler chickens. British Poultry Science 33: 821-824.

de Coca-Sinova, A., Valencia, D.G., Jiménez-Moreno, E., Lázaro, R. and Mateos, G.G., 2008. Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. Poultry Science 87: 2613-2623.

Cowieson, A.J. 2005. Factors that affect the nutritional value of maize for broilers. Animal Feed Scienece and Technology 119: 293-305.

Dalibard, P. and Paillard, E., 1995. Use of the digestible amino acid concept in formulating diets for poultry. Animal Feed Science and Technology, 53: 189-204.

Dari, R.L., Penz Jr., A.M., Kessler, A.M. and Jost, H.C., 2005. Use of digestible amino acids and the concept of ideal protein in feed formulation for broilers. Journal of Applied Poultry Research 14: 195-203.

Douglas, M.W., Parsons, C.M., 1999. Dietary formulation with rendered spent hen meals on a total amino acid versus a digestible amino acid basis. Poultry Science 78: 556-560.

Dänicke, S., Dusel, G., Jeroch, H. and Kluge, H., 1999. Factors affecting efficiency of NSP-degrading enzymes in rations for pigs and poultry. Agribiological Research 52: 1-24.

Eklund, M., Mosenthin, R., Piepho, H.P. and Rademacher, M., 2008. Estimates of basal ileal endogenous losses of amino acids by regression analysis and determination of standardised ileal amino acid digestibilities from casein in newly weaned pigs. Journal of the Science of Food and Agriculture 88: 641-651.

Flores, M.P., Castañon, J.I.R. and McNab, J.M., 1994. Nutritive value of triticale fed to cockerels and chicks. British Poultry Science 35: 527-536.

Frikha, M., Serrano, M.P., Valencia, D.G., Rebollar, P.G., Fickler, J. and Mateos, G.G, 2012. Correlation between ileal digestibility of amino acids and chemical composition of soybean meals in broielrs at 21 days of age. Animal Feed Science and Technology 178: 103-114.

Gallardo, C., Dadalt, J.C., Kiarie, E. and Trindade Neto, M.A. 2017. Effects of multi-carbohydrase and phytase on standardized ileal digestibility of amino acids and apparent metabolizable energy in canola meal fed to broiler chicks. Poultry Science 96: 3305-3313.

Golian, A., Guenter, W., Hoehler, D., Jahanian, H. and Nyachoti, C.M., 2008. Comparison of various methods for endogenous ileal amino acid flow determination in broiler chickens. Poultry Science 87: 706-712.

Green, S., Bertrand, S.L., Duron, M.J.C. and Maillard, R., 1987. Digestibilities of amino acids in maize, wheat and barley meals, determined with intact and caecetomised cockerels. British Poultry Science 28: 631-641.

Green, S. and Kiener, T., 1989. Digestibilities of nitrogen and amino acids in soyabean, sunflower, meat and rapeseed meals measured with pigs and poultry. Animal Production 48: 157-179.

Gruhn, K., Hennig, A. and Zander, R., 1989. Unterschungen zur Verdaulichkeit der Rohnährstoffe und Aminosäuren der Gerste und des Hafers mit kolostomierten Henne und Hähnen. Archiv für Geflügelkunde 53: 151-156.

Gutierrez-Alarmo, A., De Ayla, P.P., Verstegen, M-W.A., Den Hartog, L.A. and Willamide, M.J., 2008. Variability in wheat. Factors affecting ist nutritional value. World's Poultry Science Journal 64: 20-39.

Hesselman, K., Elvinger, K., Nilsson, M. and Thomke, S., 1981. The effect of β-glucanase supplementation, stage of ripeness and storage treatments of barley in diets fed to broiler chickens. Poultry Science 60: 2664-2671.

Hoehler, D., Lemme, A., Ravindran, V., Bryden, W.L. and Rostagno, H.S. 2006. Feed formulation in broiler chickens based on standardized ileal amino acid digestibility. P. 197-212. In: VIII Simposium Internacional de Nutrición Acuicola. Monterrey, Nuevo León, México.

Huang, K.H., Li, X., Ravindran, V. and Bryden, W.L., 2006. Comparison of apparent ileal amino acid digestibility of feed ingredients measured with broilers, layers and roosters. Poultry Science 85: 625-634.

Hughes, R. J. and Choct, M. 1999. Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry. Aust. J. Agric. Res. 50: 689-701.

Kadim, I.T. and Moughan, P.J., 1997. Ileal amino acid digestibility assay for growing meat chicken – effect of the imposition of a fasting period and the nature of the test diet. British Poultry Science 38: 285-290.

Kadim, I.T., Moughan, P.J. and Ravindran, V., 2002. Ileal amino acid digestibility assay for the growing meat chicken – comparison of ileal and excreta amino acid digestibility in the chicken. British Poultry Science 44: 588-597.

Kasprzak, M.M., Houdijk, J.G.M., Kightley, S., Olukosi, O.A., White, G.A., Carre, P. and Wiseman, J., 2016. Effects of rapeseed variety and oil extraction method on the content and ileal digestibility of crude protein and amino acids in rapeseed cake and softly processed rapeseed meal fed to broiler chickens. Animal Feed Science and Technology 213: 90-98.

Kasprzak, M.M., Houdijk, J.G.M., Olukosi, O.A., Appleyard, H., Kightley, S.P.J, Carre, P. and Wiseman, J., 2017. The influence of oil extraction process of different rapeseed varieties on the ileal digestibility of crude protein and amino acids in broiler chickens. Animal Feed Science and Technology 227: 68-74.

Kaczmarek, S.A., Hejdysz, M., Kubiś, M., Nowaczewski, S., Mikuła, R. and Rutkowski, A., 2019. Effects of feeding intact, ground and/or pelleted rapeseed on nutrient digestibility and growth performance of broiler chickens. Archives of Animal Nutrition. Published online 18 Dec 2019. Pp. 1-15.

Khajali, F. and Slominski, A., 2012. Review: Factors that affect the nutritive value of canola meal for poultry. Poultry Science 91:2564-2575.

Klose, C. and Arendt, E.K., 2012. Proteins in oats; their synthesis and changes during germination: A Review. Critical Reviews in Food Science and Nutrition 52: 629-639.

Kong, C. and Adeola, O., 2013. Additivity of amino acid digestibility in corn and soybean meal for broiler chickens and White Peckin ducks. Poultry Science 92: 2381-2388.

Lemme, A., Ravindran, V. and Bryden, W.L., 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World´s Poultry Science Journal 60: 423-437.

Lien, K.A., Sauer, W.A. and Fenton, M., 1997. Mucin output in ileal digesta of pigs fed a protein-free diet. Journal of Animal Science 72: 1737-1743.

Luke. 2020a. Feed tables and nutrient requirements. Natural Resources Institute Finland, Helsinki, Finland. [cited 1.1.2020]. Available at: luke.fi/feedtables

Luke 2020b. Meat production annually. Poultry total. Natural Resources Institute Finland, Helsinki, Finland. [cited 1.1.2020]. Available at: stat.luke.fi

Maiorka, A., Dahlke, F., Santin, E., Kessler, A.M. and Penz Jr., A.M., 2004. Effect of energy levels of diets formulated on total or digestible amino acid basis on broiler performance. Brazilian Journal of Poultry Science 6: 87-91.

McCracken, K.J. and Quintin, G., 2000. Metabolisable energy content of diets and broiler performance as affected by wheat specific weight and enzyme supplementation. British Poultry Science 41: 332-342.

Mossé, J., Huet, J.C. and Baudet, J., 1985. The amino acid composition of wheat grain as a function of nitrogen content. Journal of cereal science 3: 115-130.

Newkirk, R.W., Classen, H.L. and Edney, M.J., 2003a. Effects of prepress-solvent extraction on the nutritional value of canola meal for broiler chickens. Animal Feed Science and Technology 104: 111-119.

Newkirk, R.W., Classen, H.L., Scott, T.A. and Edney, M.J., 2003b. The digestibility and content of amino acids in toasted and non-toasted canola meals. Canadian Journal of Animal Science 83: 131-139.

NRC 1994. Nutrient requirements of poultry. 9th revised edition, 1994. National Research Council. Subcommittee on poultry nutrition. National Academy Press, Washington, USA 1994.

Osho, S.O., Babtunde, O., O., Adeola, O., 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dries grains with solubles in mixed diets fed to broilers. Poultry Science 98: 1333-1340.

Parsons, C.M. 1996. Digestible amino acids for poultry and swine. Animal Feed Science and Technology 59: 147-153.

Peltonen-Sainio, P., Kontturi, M., Rajala, A., 2004. Impact dehulling oat grain to improve quality of on-farm produced feed 1. Hullability and associated changes in nutritive value and energy content. Agricultural and Food Science 13: 18-28.

Perttilä, S., Valaja, J., Partanen, K. and Jalava, T., 2001. Effect of volume-weight on apparent ileal and excreta amino acid digestibility and feeding value of barley for poultry. Journal of Animal and Feed Sciences10: 671-685.

Pettersson, D., Graham, H. and Åman, P., 1991. The nutritive value for broiler chickens of pelleting and enzyme supplementation of a diet containing barley, wheat and rye. Animal Feed Science and Technology 33: 1-14.

Poureslami, R., Batal, A.B. and Jung, B., 2012. Effect of ileal sub-section and the method of collection of digesta on the determination of apparent ileal digestibility of amino acids in broilers. Animal Feed Science and Technology 177: 130-133.

Ravindran, V., Abdollahi, M.R. and Bootwalla, S.M., 2014. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poultry Science 93: 2567-2577.

Ravindran, V., Adeola, O., Rodehutscord, M., Kluth, H., van der Klis, J.D., van Eerden, E. and Helmbrecht, A., 2017. Determination of ileal digestibility of amino acids in raw materials for broiler chickens – Results of collaborative studies and assay recommendations. Animal Feed Science and Technology 225: 62-72.

Ravindran, V., Hendriks, W.H., Camden, B.J., Thomas, D.V., Morel, P.C.H. and Butts, C.A., 2002. Amino acid digestibility of meat and bone meals for broiler chickens. Australian Journal of Agricultural Research 53: 1257-1264.

Ravindran, V., Hew, L. I., Ravindran, G. and Bryden, W. L. 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. British Poultry Science 40: 266-274.

Ravindran, V., Hew, L. I., Ravindran, G., Bryden, W. L., 2004. Endogenous amino acid flow in the avian ileum: quantification using three techniques. British Journal of Nutrition 92: 217-223.

Rodehutscord, M., Rükert, C., Maurer, H.P., Schenkel, H., Schipprack, W., Bach Knudsen, K.E., Schollenberg, M., Laux, M., Eklumd, M., Siegert, W. and Mosenthin, R., 2016. Variation in chemical composition and physical characteristics of cereal grains from different genotypes. Archives of Animal Nutrition 70: 87-107.

Rostagno, H.S. and Pupa, J.M.R., 1995. Diet formulation for broilers based on total versus digestible amino acids. Journal of Applied Poultry Research 4: 293-299.

Rundgren, M., 1988. Evaluation of triticale given to pigs, poultry and rats. Animal Feed Science and Technology 19: 359-375.

Rutherfurd, S.M., Chung, T.K., Moughan, P.J., 2007. The effect of a commercial enzyme preparation on apparent metabolizable energy, the true ileal amino acid digestibility, and endogenous ileal lysine losses in broiler chickens. Poultry Science 86: 665-672.

Scott, T. A., Silversides, F. G., Classen, H. L., Swift, M. L. and Bedford, M. R. 1998. Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent digestible energy of wheat and barley. Poultry Science 77: 456-463.

Schipper, A., 1984. Verändrung der Proteinzusammensetzung in Weizen- und Gerstenkörnern wärend der Reife. 1984. Angewande Botanik 58: 401-422.

Short, F. J., Wiseman, J. and Boorman, K. N., 1999. Application of a method to determine ileal digestibility in broilers of amino acids in wheat. Animal Feed Science and Technology 79: 195-209.

Sibbald, I.R., 1979. A bioassay for available amino acids and true metabolizable energy in feedinsgtuffs. Poultry Science 58: 668-673.

Siegert, W., Boguhn, J., Maurer, H.P., Weiss, J., Zuber, T., Möhring, J. and Rodehutscord, M. 2017. Effect of nitrogen fertilisation on the amino acid digestibility of different triticale genotypes in caecetomised laying hens. Journal of Science of Food and Agriculture 97: 144-150.

Siriwan, P., Bryden, W.L., Mollah, Y. and Annison, E.F., 1993. Measurement of endogenous amino acid losses in poultry. British Poultry Science 34: 939-949.

Summers, D.J. and Robblee, A.R., 1985. Comparison of apparent amino acid digestibilities in anesthetized versus sacrifice chickens using diets containing soybean meal and canola meal. Poultry Sciene 64: 536-541.

Szczurek, W., 2009. Standardized ileal digestibility of amino acids from several cereal grains and protein-rich feedstuffs in broiler chickens at the age of 30 days. Journal of Animal and Feed Sciences 18: 662-676.

Szczurek, W., 2010. Standardized ileal digestibility of amino acids in some cereals, rapeseed products and maize DDGS for broiler chickens at the age of 14 days. Journal of Animal and Feed Sciences 19: 73-81.

Svihus, B., Herstadt, O. and Newman, C.W., 1997a. Effect of high-moisture storage of barley, oats and wheat on chemical content and nutritional value for broiler chickens. Acta Agriculturae Scandinavica, 47: 39-47.

Svihus, B., Newman, C.W., Newman, R.K. and Selmer-Olsen, I., 1997b. Changes in extract viscosity, amino acid content, and soluble and insoluble β-glucan and dietary fibre content of barley during different high-moisture storage conditions. Animal Feed Science and Technology, 64: 257-272.

Svihus, B., Seslmer-Olsen, I. and Båthen, E., 1995. Effect of different preservation methods for high-moisture barley on feeding value for broiler chikens. Acta Agricuturae Scandinavica 45: 252-259.

Tahir, M. and Pesti, G.M., 2012. A comparison of digestible amino acid databases: Relationship between amino acid concentration and digestibility. The Journal of Applied Poultry Research 21: 1-12.

Ten Doeschate, R.A.H.M., Scheele, C.W., Schreurs, V.V.A.M. and Van der Klis, J.D., 1993. Digestibility studied in broilers chicks: Influence of genotype, age, sex and method of determination. British Poultry Science 34: 131-146.

Titus, H. W., Mehring, A. L., Johnson, D. Jr., Nesbitt, L. L. Jr. and Tomas, T., 1959. An evalution of MCF (Micro-Cel-Fat), a new type of fat product. Poultry Science 38: 1114-1119.
Toghyani, M., Rodgers, N., Iji, P.A. and Swick, R.A., 2015. Standardized ileal amino acid digestibility of expeller-extracted canola meal subjected to different processing conditions for starter and grower broiler chickens. Poultry Science 94: 992-1002
Woyengo, T.A., Kiarie, E. and Nyachoti, C.M., 2010. Metabolizable energy and standardised ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chickens. Poultry Science 89: 1182-1189.
Wu, Y.B., Ravindran, V. and Hendriks, W.H., 2003. Effects of microbial phytase, produced by solid-state fermentation, on the performance and nutrient utilisation of broilers fed maize- and wheat-based diets. British Poultry Science 44: 710-718.
Zuber, T., Maurer, H.P., Möhring, J., Nautscher, N., Siegert, W., Rosenfelder, P. and Rodehutscord, M., 2017. Variability in amino acid digestibility of triticale grain from diverse genotypes as studied in cecectomized laying hens. Poultry Science 95: 2861-2870.

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