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Dietary factors affecting hindgut protein fermentation in broilers: a review

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High growth rates in modern-day broilers require diets concentrated in digestible protein and energy. In addition to affecting feed conversion efficiency, it is important to prevent surplus dietary protein because of greater amounts of undigested protein entering the hindgut that may be fermented by the resident microbiota. The latter may result in increased formation of a wide range of protein-derived compounds including ammonia, amines, indoles and phenols, in addition to secondary products (lactate, succinate) and gases such as methane, hydrogen and carbon dioxide. In poultry, studies have shown the presence of protein fermentation products such as biogenic amines and branched chain fatty acids (BCFA) in the ileal and caecal digesta. The production and metabolism of nitrogenous waste products (as a result of protein fermentation) such as uric acid and ammonia may lead to a burden on the organism and cause additional energy losses. Although biogenic amines are important for normal gut development, greater concentrations may cause gizzard erosion, mortality and depressed growth rate in broilers. A decrease in indigestible protein reduces hindgut protein fermentation. In broilers, feeding diets with coarse particles (between 600 to 900 µm) and/or using feed additives, such as pre- and probiotics and organic acids, especially butyric acid, may improve protein digestion, thereby, potentially reducing hindgut protein fermentation. Studies are therefore needed to determine the extent and importance of hindgut protein fermentation on performance and gut health in broilers.

Keywords: gut health; protein fermentation; hindgut; broilers; feeding strategy; organic acids

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Introduction

The length of the growth period in broilers is decreasing year by year. Currently meat chickens can attain 2.0 kg body weight after consuming 3.0 kg of feed within a 5 week period (Choct, 2009). This rapid growth rate is related to a very high capacity for protein deposition combined with a high feed intake as a result of genetic selection over the past decades (Havenstein *et al.*, 2003). Rapid growth requires diets that have a high content of digestible protein and energy. In practical feed formulation diets high in digestible protein can contain appreciable quantities of undigested protein. The latter protein enters the hindgut where it can be used as a substrate for microbial degradation (Jeaurond *et al.*, 2008). This undigested protein and unabsorbed endogenous protein may promote the proliferation of microbiota which uses the undigested amino acids as an energy source (Reid and Hillman, 1999). The latter can occur especially if insufficient fermentable carbohydrates are available. In an ideal situation, the unabsorbed dietary and endogenous amino acids in the large intestine will serve as building blocks for microbial protein synthesis with dietary carbohydrates used as an energy source. The extent to which hindgut protein fermentation occurs consequently depends on the availability of protein in relation to the amount of fermentable carbohydrates as these carbohydrates are a preferred source of energy for gut microbes (Rehman *et al.*, 2008). As such, the digestibility of dietary protein and carbohydrates, and their dietary inclusion level are important to determine the amounts of protein and carbohydrates entering the hindgut and thus the potential for protein and/or carbohydrate fermentation (Gill and Rowland, 2002).

Proteolysis is the first step in the utilisation of protein by the microbiota (Jeaurond *et al.*, 2008). Subsequent deamination and decarboxylation of amino acids delivers a substrate which can be used as an energy source. In poultry, caeca are the major sites for fermentation because they provide a stable environment for indigenous microbiota (Meyer *et al.,* 2013) which may be due to a longer residence time of digesta there. Besides using dietary and endogenous amino acids for energy and protein synthesis, gut microbiota can produce different putrefactive compounds from undigested nitrogen (N) including indoles, phenols, sulphur-containing compounds, ammonia and amines. In addition to the beneficial products like volatile fatty acids (VFA) and branched chain fatty acids (BCFA), some secondary products (lactate, succinate) and several gases such as methane, hydrogen, hydrogen sulphide (H_2S), and carbon dioxide (Macfarlane *et al.*, 1992) can be produced.

The impact of protein fermentation on performance and gut health of modern-day broilers is becoming increasingly relevant in view of the growing demand for dietary protein sources which often have a low digestibility by poultry. The trend to search for cheaper sources of feed protein is due to the ever increasing price of highly digestible protein ingredients and the aim of cost-effective production. The goal of this review paper is to ascertain the occurrence of hindgut protein fermentation and its impact on broiler performance. Secondly, this review aims to describe the possibilities of reducing hindgut protein fermentation in broilers through the application of a number of potential nutritional strategies. Due to a lack of information in poultry nutrition literature, some examples of other species are used in this review.

Protein fermentation in broilers

Fermentation is a breakdown of organic products by microbiota in the GIT. Fermentation of carbohydrates is considered beneficial because it results in the production of VFAs

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which are used as an energy source (Guo *et al.*, 2003), while protein fermentation is generally considered detrimental for health. The latter is due to the production of toxic compounds such as amines and ammonia (Macfarlane *et al.*, 1988), and may result in poor performance of the birds. Rehman *et al.* (2007) reported the presence of lactate, ammonia and SCFA in the crop content of broilers, indicating that fermentation of dietary components can occur in the crop. In poultry, due to a short residence time and low pH of digesta in the duodenum, there is a little microbial activity in the duodenum (Rehman *et al.*, 2007). The SCFA concentration due to microbial activity is, therefore, low (2-12 μmol/ g digesta) or sometimes even undetectable in the digesta in this gut segment (Rehman *et al.*, 2007). In the caeca, bacterial fermentation activity can be high due to a long residence time of digesta and a high microbial density (Guo *et al.*, 2003). To our knowledge, there are no studies in broilers that specifically studied the occurrence and importance of hindgut protein fermentation. In a number of studies, however, several metabolites, which are indicative of hindgut protein fermentation in broilers, have been quantified.

GUT MICROBIOTA POPULATIONS

The gut microbiota ecosystem is complex and is composed of fungi, protozoa and, most important, bacteria (Gabriel *et al.*, 2005). Numbers of known bacterial species in the chicken guts has increased from 200 up to 640 in recent years (Apajalahti *et al.*, 2004) due to advances in new techniques of microbiota analysis. The microbiome is affected by numerous factors including diet (Torok *et al.*, 2011), genetics of the host (Mignon-Grasteau *et al.,* 2004), age of the bird and housing condition (Gabriel *et al.*, 2005). Microbiota diversity in different parts of the GIT reflects the composition of the substrate present in that intestinal segment as microbiota species differ in their requirements (Kiarie *et al.*, 2013). If the growth of pathogenic microbiota in the small intestine is increased, this may enhance endogenous losses and increase the maintenance requirements for replacing these losses thus compromising growth efficiency (Ferrell, 1988). A high inflow of undigested material in the large intestine allows microbes to use the digesta as a substrate in order to proliferate. The gut microbial community is influenced by dietary protein source favouring the growth of pathogenic bacteria like *Campylobacter spp.* (Wise and Siragusa, 2007) and *C. perfringens* (Wilkie *et al.*, 2005). Similarly, increasing the level of dietary protein resulted in a higher *C. perfringens* counts independent of the protein source used (Drew *et al.*, 2004). There is evidence in broilers that glycine (at levels above 3% in the diet) supports the growth of *C. perfringens* in ileum and caecum (Dahiya *et al.*, 2005). An overview of different microbiota populations throughout the GIT of poultry is presented in *Table 1*.

At day one after hatching, digesta from the ileum contains approximately 10^8 bacteria per gram and this increases to 10^9 at day three of age, whereafter it remains constant till 30 days of age in broilers (Apajalahti *et al.*, 2004). *Lactobacillus* (70%) is the major species found in the ileum, whereas the other species include *Clostridiaceae* (11%), *Streptococcus* (6.5%) and *Enterococcus* (6.5%) (Lu *et al.,* 2003). This population is influenced by dietary ingredients such as medium chain fatty acids that decreased the growth of gram positive *Firmicutes* and some other species including *Lactobacillus*, *Micrococcaceae* and *Enterococcaceae*, whereas the growth of gram negative bacteria is increased (Van Der Hoeven-Hangoor *et al.,* 2013). The latter authors related the change in microbiota population with higher dietary concentrations of medium chain fatty acids to the sensitivity of gram positive compared with gram negative bacteria. In the caeca there is a higher diversity of microbiota compared to the ileum, with dominance of *Clostridium* (*Ruminococcus, Eubacterium*) species (Gabriel *et al.*, 2005). The microbiota population in the caeca of broilers has been estimated at 10^{11} bacterial

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numbers per gram of caecal digesta and to mainly include obligate anaerobes (Barnes *et al.*, 1972). In broilers, during the first few days after hatch, *Enterococcus faecalis* is usually dominant in caeca, but obligatory anaerobes become dominant as the bird grows. The caecal microbial population at 14 days of age contains anaerobic species comprising of gram positive (*Clostridium spp.*, *Eubacterium spp., Lactobacillus spp.*, anaerobic cocci) and gram negative (*Bacteriodes spp*., *Fusobacterium spp*. and *Gemmiger spp.*) bacteria (Barnes *et al.*, 1972). This microbiota population reached a steady stage after 40 days of age and at this age consists mainly of gram positive bacteria including *Bifidobacterium spp., Clostridium spp., Cocci, Streptococcus spp., E. coli*, *Bacteriods spp.* and *Lactobacillus spp.* (Barnes *et al.*, 1972).

DIFFERENCES BETWEEN ILEAL AND EXCRETA DIGESTIBILITY

Nitrogen metabolism in the hindgut includes both catabolism and synthesis of proteins from dietary, endogenous and microbial origin as well as other N-containing compounds. Although there is some absorption of amino acids in the large intestine of monogastric animals, quantitatively this is insignificant compared to absorption in the small intestine (Hendriks *et al.*, 2012). Dietary amino acids recovery in excreta decrease as a result of net protein degradation in the large intestine (in the form of different compounds *e.g.* biogenic amines, ammonia, indole, phenols, cresol and skatole), often causing an overestimation of amino acid digestibility. In contrast, the opposite will be the case, with amino acid digestibility being underestimated when synthesis results in a net increase of microbial amino acids in the hindgut. Kadim *et al.* (2002) did not find any difference in endogenous amino acid losses (except for Asp and Glu) when measured in ileal digesta or in excreta while comparing the ileal and excreta amino acid digestibility of different feed ingredients in broilers. The latter indicates that the large intestine does not appear to make a significant contribution to overall gut endogenous amino acid losses in broilers. As there is no significant absorption of amino acids in the hindgut (Hendriks *et al.,* 2012), the difference between ileal and excreta digestibility can be an important indicator of the occurrence of protein fermentation. If no protein fermentation occurs, digestibility values (when measured using ileal digesta) should not be significantly different from values determined in excreta, whether apparent or standardised digestibility values. There are a number of studies indicating significant differences between ileal and excreta digestibility values of different amino acids of various feed stuffs in broilers (Sebastian *et al.,* 1997; Ravindran *et al.*, 1999; Kadim *et al.*, 2002). Some studies showed a net lower amino acid and total N recovery in the excreta compared with the ileal digesta (Sebastian *et al.,* 1997; Ravindran *et al.,* 1999), whereas others showed an increase in amino acid recovery in the excreta (Ten Doeschate *et al.,* 1993; Kadim *et al.,* 2002). The difference between ileal and excreta digestibility values of various amino acids of different feed ingredients for poultry are shown in *Table 2*. For most amino acids, negative values are observed, although the range within an ingredient can be highly variable *e.g.* wheat. The positive values for amino acids such as valine, methionine, isoleucine, phenylalanine and alanine indicate a net synthesis of these amino acids in the poultry hindgut. However, the negative values of amino acids such as threonine, leucine, histidine, lysine, arginine, aspartic acid, serine, glutamic acid, cysteine, proline, and tyrosine indicate a net catabolism of these amino acids in the large intestine of poultry. This may result in the formation of biogenic amines and BCFA which are discussed in detail later in the review.

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Metabolites of hindgut protein fermentation

The main function of the GIT is the digestion and absorption of nutrients (Fasano and Shea-Donohue, 2005), the possibility for fermentation of indigestible ingredients such as NSP and proteins (Niba *et al.*, 2009) and the excretion of waste products resulting from the digestive process (Cummings, 1983) and metabolism. The end products of protein fermentation by resident microbiota in the hindgut are generally considered detrimental for the host animal (Nollet *et al.*, 1999). Protein fermentation has been associated with the production of biogenic amines, BCFA, ammonia, phenols, indoles, cresol, skatole and hydrogen sulphide (H_2S).

BIOGENIC AMINES

Amines are produced by decarboxylation of amino acids (Urlings *et al.*, 1992) by intestinal microbiota such as *Bacteroides*, *Clostridium*, *Bifidobacterium*, *Enterobacterium* and *Streptococcus spp.* (Allison and Macfarlane, 1989). The amines formed by living organisms (biogenic amines) include monoamines (tyramine) as well as polyamines such as cadaverine, putrescine, and spermine (Larqué *et al.*, 2007). Fermentation of the amino acids histidine, ornithine, lysine, methionine, tyrosine, phenylalanine, tryptophan, and arginine results in the production of histamine or spermidine, putrescine or spermidine, cadaverine, spermidine, tyramine, phenylethylamine, tryptamine or serotonin, and agmatine or putrescine or spermidine, respectively. The polyamine, spermidine is formed from catabolism of amino acids including histidine, ornithine, methionine, and arginine. Spermidine may subsequently be converted into spermine. Polyamines, including putrescine, spermidine, and spermine, have been shown in rats to be beneficial protein catabolites required for repair of intestinal mucosal cells (Wang and Johnson, 1990). There is a scarcity of published data regarding the physiological role of biogenic amines in poultry.

Dietary supplementation of synthetic biogenic amines may result in a significant depression in growth and increased mortality in broilers. Shifrine *et al.* (1960) studied the dose response of dietary supplementation of histamine by feeding 0.25, 0.50 and 1.0% histamine in the diet. These authors reported a dose dependent decrease in the performance and an increase in proventriculus enlargement. Addition of histamine (2.2 mg/kg of feed) to a broiler diet resulted in gizzard erosion, high mortality and depressed growth rate (Fossum *et al.*, 1988). In contrast, putrescine supplementation at 0.2 and 0.4% of the diet resulted in a greater growth rate and improved feed efficiency in broilers compared with those on a diet without supplementation of putrescine, whereas supplementation of putrescine above 0.4% of the diet resulted in a decreased feed intake and poor feed conversion ratio (Smith, 1990). Similarly, Barnes *et al.* (2001) reported that supplementation of 0.1 and 0.2% histamine addition to broiler diets resulted in a 6.2 and 9.2% decrease in body weight gain, respectively. These authors, furthermore, reported a poorer feed conversion ratio and lesions in the proventriculus of birds fed histamine supplemented diets. Some studies indicated that dietary supplementation of histamine resulted in increased gastric acid secretion, leading to enlargement of the proventriculus (Shifrine *et al.*, 1960; Harry *et al.*, 1975). Tiihonen *et al.* (2010) studied the effects of essential oils on broiler performance and gut microbiota and reported an increased mortality at day 20 with a high concentration of total ileal biogenic amines biogenic amines (tryptamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine). In contrast, the authors reported improved body weight gain at day 41 with a high concentration (3150 *vs.* 2893 nmol/g wet weight) of total caecal biogenic amines (methylamine, tryptamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) in broilers fed diets supplemented with essential oil

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compared with those fed the control diet. In addition, an inverse relation between the total biogenic amine concentration in the ileal digesta and the body weight of the broilers was observed at 41 day of age. Tiihonen *et al.* (2010) reported a higher concentration of total biogenic amines (methylamine, tryptamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) in the caecum compared with ileal digesta (2893 *vs.* 776 nmol/kg wet digesta) which is consistent with higher microbial proteolytic activity. This increased activity may be due to a longer time period of digesta and/or also due to a greater microbial population in the caeca. Likewise, Rehman *et al.* (2008) reported a 9.5% greater caecal total biogenic amine concentration (tryptamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine) compared with ileal digesta in broilers fed a corn-soya diet. These authors reported greater concentrations of putrescine, cadaverine and tyramine in the ileal compared with the caecal digesta, whereby caecal samples contained approximately 4.4 times higher concentrations of spermidine. The latter results suggested greater fermentation of ornithine or arginine, lysine and tyrosine in the ileum compared to the caecum, whereas greater fermentation of methionine or histidine or ornithine or arginine was observed in the caecum compared to the ileum.

BRANCHED CHAIN FATTY ACIDS

The degradation of valine, isoleucine, and leucine in the intestinal tract of animals can results in the formation of the BCFAs isobutyrate, 2-methyl butyrate, and isovalerate, respectively. These BCFA have a pungent odour in comparison to straight short chain fatty acids which are formed from carbohydrate fermentation (Mackie *et al.*, 1998). The BCFA in the hindgut are produced by many bacteria including *Bacteroides spp.*, *Propionibacterium spp.*, *Streptococcus* and *Clostridium spp.* (Rist *et al.*, 2011). The presence of n-valerate and BCFA (1.0 mol% of each) in caecal digesta and their absence in the crop and gizzard of broilers (Rehman *et al.,* 2008) is indicative of proteolytic fermentation in the caeca and a lack thereof in the crop and gizzard. Tiihonen *et al.* (2010) reported approximately 12.9 times greater total BCFA concentrations in the caeca than the ileal digesta in broilers at 41 days of age. These authors found that isobutyric, isovaleric and 2-methyl butyric acid comprised 50.7, 29.2 and 20.1% of the total BCFA, respectively. This indicated a higher fermentation rate of valine, leucine and isoleucine in the caeca compared to the ileum. Greater levels of BCFA (19.7 *vs.* 9.5 mg acetic acid equivalents/g DM) have been reported by Guo *et al.* (2003) in intact mushroom contents compared with their polysaccharides extracts while studying the *in vitro* fermentation characteristics using broiler caecal contents. The intact mushroom had a greater protein contents (155.5 *vs.* 80.5 g/kg DM) compared with their polysaccharide extracts. The greater concentration of BCFA indicated a higher proteolytic fermentation capability from the intact material compared with the extracts, as BCFA are the only end products of protein fermentation (Macfarlane *et al.,* 1992). Similarly, Faber *et al.* (2012) reported that broilers fed a soybean meal (SBM) diet had 2.7, 2.3, 3.0 and 2.5 fold greater isobutyrate, isovalerate, valerate and total BCFA compared with those fed a soy protein isolate (SPI) diet. These data indicated that there is higher protein fermentation in protein rich diets in the caeca. Meyer *et al.* (2012) reported greater caecal molar ratios of isobutyrate and isovalerate (0.4 *vs*. 0.1 and 0.1 *vs.* 0.3 mol%, respectively) in layers fed 5% feather supplemented diets compared with a control and attributed this greater concentration to protein fermentation of the dietary supplemented feathers in the caeca. The concentration in the ileum was significantly lower compared with the caecal concentration. Similarly, in another study, Meyer *et al.* (2013) reported approximately 19% greater SCFA concentrations in the caecal compared with the ileal digesta in high compared with low feather pecking breeds. These authors, however,

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found significantly greater molar percentages of caecal isobutyric and isovaleric acid (1.1 *vs.* 0.7 and 1.3 *vs.* 0.6, respectively) in low compared with high feather pecking breed. The later metabolites were not detectable in the ileal digesta. This greater concentration of BCFA (isovaleric and isobutyric acid) in the caeca suggested enhanced microbial protein catabolic activity in the caeca compared with the ileum.

AMMONIA

Ammonia is produced by deamination of amino acids and is a toxic waste product of microbial fermentation. It may be absorbed and excreted as uric acid by birds (Salter and Fulford, 1974) or transformed into bacterial protein by the microbiota (Rist *et al.*, 2011). According to Nousiainen (1991), a high concentration of ammonia in the large intestine of pigs potentially reflects a large quantity of undigested protein entering the hindgut which can negatively affect the growth of intestinal epithelial cells. This ammonia can pass through the gut wall and disturb intestinal mucosal development, as shown by reduced villus height. The absorption of ammonia from the gut wall has toxic effects on enterocytes (Macfarlane *et al.*, 1992). Higher caecal ammonia levels resulted in an increase in caecal pH, providing a favourable environment for pathogenic microbiota and an increased risk for enteritis (Abdl-Rahman *et al.,* 2011). Changes in blood ammonia levels and excreta characteristics due to lowering the dietary CP levels in broilers have been studied by Namroud *et al.* (2008). They found a decrease in faecal nitrogen from 50.3 to 36.3 mg/g DM and uric acid from 113.5 to 101.1 mg/g DM of excreta digesta by lowering the dietary CP from 23 to 17%. The supplementation of 10% additional essential amino acids (Lys, Thr, Arg, and Trp) to a low (17%) CP diet resulted in an increased ammonia level (0.71 *vs*. 0.68 mg/100 ml) in the blood compared to the low CP diet without supplementation of essential amino acids. There can be a negative correlation between ammonia level and growth performance, as reported in rats (Lardy and Feldott, 1950) and dogs (Russek, 1970). Ammonia is mainly converted into uric acid and the high levels of ammonia in blood may be reduced by enhancing the conversion of ammonia into uric acid, which requires 1 M of glycine to convert each molecule of uric acid in birds (Namroud *et al.,* 2008). This may lead to amino acid loss resulting in poor growth performance. High concentration of ammonia in the blood will result in more ammonia-nitrogen in the faeces, because there is a strong correlation between the two. A greater amount of blood ammonia resulted in reduced BW, feed intake, poor FCR and greater liver weight (3.2 *vs*. 2.4 % of the weight of visceral organs) in broiler fed a low (17%) CP diet supplemented with essential amino acids compared with broilers fed a high (23%) CP diet (Namroud *et al.,* 2008). The authors proposed that lower BW and greater liver weight may be due to increased liver uric acid metabolism. In mammals, greater activity of urea cycle enzymes inhibits growth rate (Schimke, 1963). The results from an *in vitro* fermentation study using mushrooms, medicinal plants and their polysaccharide fractions by Guo *et al.* (2003) using broiler caecal contents showed a significantly greater ammonia level (370.6 *vs*. 249.3 mg/l) for the intact materials than for the extracts due to a greater protein and lower carbohydrate contents of the intact materials compared with their extracts. These authors, furthermore, reported a positive correlation between ammonia and BCFA in caecal digesta. Higher levels of ammonia indicated increased protein and decreased carbohydrate fermentation of the intact material compared with the extracts. Greater levels of dietary indigestible protein, especially in the absence of proper levels of fermentable carbohydrates, should be avoided for optimum performance and gut health of the birds (Guo *et al.,* 2003). Similarly, Khempaka *et al.* (2011) conducted a study to evaluate the effects of chitin (purified 83.9% chitin) addition at 1.07, 2.26, 3.34, and 4.53% of diet and four different levels of shrimp meal (SM; 5, 10, 15, and 20%) on growth performance, intestinal microbial populations, VFA, and

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ammonia production in broilers. These authors reported greater concentrations of ammonia in the caecal compared with the ileal digesta (0.67 *vs*. 0.13 g/100g of digesta) in broilers fed 15% SM indicating more proteolytic activity in the caecum. These authors also assessed blood urea N and found the lowest concentration (1.87 *vs.* 2.15 mg/dl) in broilers fed 15% SM compared with the control group which resulted in improved performance of the broilers compared with other groups.

PHENOLS, INDOLES, CRESOL AND SKATOLE

Bacterial fermentation of aromatic amino acids such as phenylalanine, tryptophan, histidine and tyrosine can result in the production of phenols and indoles (Windey *et al.*, 2012). These compounds are involved in carcinogenesis in the animal colon, whereas phenols are involved in nitrosation of secondary amines by nitrate (Kikugawa and Kato, 1988) and indoles can also enhance this nitrosation (Zuccato *et al.*, 1993). Aerobic metabolism of aromatic amino acids results in the production of skatole, cresol and other phenolic compounds (Elsden *et al.*, 1976). Skatole and methyl sulphide are mainly responsible for the characteristic smell of excreta, whereas cresol is associated with noxious gases. Terada *et al.* (1993) reported the presence of phenol, p-cresol, indole and skatole at concentrations of 64.9, 64.1, 43.8 and 5.5 µg/g wet digesta, respectively, in the caeca of broilers at 56 days of age. The concentration of these compounds in the caecal digesta showed a decreasing trend with age of the broilers which suggests increase in protein digestibility with the age of broilers. Similarly, Terada *et al.* (1994) also conducted a study in broilers and reported the presence of phenol, p-cresol, indoles, and skatole (32.7, 91.3, 8.1, and 13.9 µg/g wet digesta, respectively) in the caecal digesta at 62 days of age. The presence of these putrefactive compounds in the caeca of broilers indicates the occurrence of fermentation of tyrosine and tryptophan, respectively. The presence of these compounds was correlated with the higher caecal population of protein fermenting microbiota such as *Clostridia*, *Bacteriodaceae*, and *Staphylococci spp.* (Terada *et al.,* 1994).

HYDROGEN SULPHIDE

Hydrogen sulphide (H_2S) is one of the end products of fermentation of dietary and mucinous sulphate and sulphur-containing amino acids, such as methionine, cysteine and taurine, by sulphate-reducing bacteria (Lewis and Cochrane, 2007). Hydrogen sulphide is a highly toxic agent for rodents, comparable to cyanide (Reiffenstein *et al.*, 1992). The toxic potential of H_2S on colonic cells involves damage of the most important energy pathway for colonocytes by disrupting butyrate oxidation (De Preter *et al.*, 2012). In broilers, sulphur amino acids, such as Met and Cys are a major source of sulphur in faeces and are related to H_2S production by microbiota (Kadota and Ishida, 1972). Chavez *et al.* (2004) reported the presence of hydrogen sulphide, dimethyl disulphide and trimethyl trisulphide 224.4, 11.5 and 3.6 ng/g faeces, respectively, in the excreta of broilers fed diets supplemented with sodium methioninate. They reported the presence of hydrogen sulphide, dimethyl disulphide and trimethyl trisulphide, 49.6, 5.6 and 2.9 ng/g of faeces, respectively, in the excreta of broilers fed the control corn-soybean based diet (CP: 21%), which may indicate the catabolism of amino acids. Similarly, Chen *et al.* (2012) reported the presence of (49 mg/l) H_2S in caecal digesta of broilers at 35 days of age, indicating that H_2S is a metabolite of protein degradation in poultry. The toxic effects of H_2S may not be as relevant in poultry as in mammals, due to the high demand of the essential S-containing amino acids including methionine and cysteine for feather production. These amino acids are, therefore, limiting in most poultry diets and are absorbed in the foregut.

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Mode of action of dietary factors to reduce hindgut protein fermentation

PROTEIN SOURCE AND INCLUSION LEVEL

Dietary CP content and its digestibility affect the formation and quantity of microbial metabolites resulting from hindgut protein fermentation (Hobbs *et al.*, 1996). Proteolytic microbial fermentation is directly related to CP and high levels of undigested dietary CP in the hindgut, which may enhance the proliferation of pathogenic microbiota in the GIT (Ball and Aherne, 1987). The percentage of undigested amino acids of commonly used ingredients in poultry diets may vary 8 to 35 of the total dietary CP content (CVB, 2007). A negative linear effect of increasing indigestible CP contents in the diet on feed conversion ratio (FCR) in broilers has been reported by De Lange *et al.* (2003). These authors found that FCR was increased by 0.080 with an increase of approximately 10 g/kg of indigestible CP contents (from 35.3 to 43.9 g/kg) of the diet using different levels of SBM, peas, hydrolysed feather meal and meat and bone meal. Highly digestible protein sources at appropriate inclusion levels in broiler diets can be expected to reduce hindgut protein fermentation. Improving foregut protein digestion, thereby, increasing protein digestion will reduce the inflow of indigested amino acids in the colon and reduce the potential for protein fermentation. Some strategies can be applied to increase the digestion and absorption of proteins in the foregut.

Total dietary CP levels, amino acid composition and digestibility determine the amount of protein arriving in the hindgut. A high number of proteolytic microbiota (such as *Campylobacter*, *Bacteroides*, *Clostridia*, and *Prevotella spp.*) has been reported in finishing barrows fed a high CP (34%) corn-SBM based diet compared with those fed a low CP (15%) diet (Anugwa *et al.,* 1989). In addition, Opapeju *et al.* (2009) reported a higher (414.9 *vs*. 182.7 mg/l) ammonia-N concentration in colonic digesta of piglets fed a high (22.5%) CP diet compared to those fed a low (17.6%) CP diet supplemented with essential amino acids. Similarly, these authors reported more carbohydrate fermenting bacteria (Roseburia) in the colonic digesta of piglets fed low CP diet and suggested that this may have shifted the microbiota population towards a more beneficial carbohydrate fermenting population. In birds, a low level of dietary CP may negatively affect performance although it can have beneficial effects on the physical conditions in the gut (*Table 3*).

Table 3 Effects of lowering dietary crude protein on digesta characteristics and performance of poultry and pigs.

Crude protein level (%)	Species	Digesta[2,3] characteristics (%)			Performance change (%)		Reference
		NH_3	VFA	BCFA	BW	FCR	
22.5[1] to 18.5	Poultry	ND	ND	ND	- 1.5	- 4.8	Laudadio *et al.* (2012)
22[1] to 10	Poultry	ND	ND	ND	- 32.7	+ 28.0	Buwjoom *et al.* (2010)
23[1] to 19	Poultry	- 15.6	ND	ND	- 17.5	+ 6.0	Namroud *et al.* (2009)
25.6[1] to 17.5	Pigs	- 19.0	- 15.3	- 1.9	- 6.7	- 2.6	Heo *et al.* (2009)
22.5[1] to 17.6	Pigs	- 66.0	+ 4.5	ND	- 5.2	- 1.9	Opapeju *et al.* (2009)
24[1] to 20	Pigs	- 32.3	- 27.5	- 52.0	- 3.5	0	Htoo *et al.* (2007)

[1]Control diet, [2]Caecal digesta in poultry, [3]Faecal digesta in pigs VFA=Volatile fatty acids, BCFA=Branched chain fatty acids, BW=Body weight, FCR=Feed conversion ratio, ND=Not determined.

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DIET STRUCTURE

There are no studies showing direct effects of dietary particle size on hindgut protein fermentation in poultry. However, there are studies from which it may be inferred that particle size has the potential to reduce hindgut protein fermentation in broilers. A number of studies have shown that more coarsely ground diet improves broilers performance and villus heights in the small intestine (*Table 4*). This improved performance was due to greater digestibility of protein because of dietary coarseness (Pacheco *et al.,* 2013). Extensive research has shown that the inclusion of a 'coarse' mash resulted in a heavier gizzard which maximised the grinding capacities of the GIT and enhances digestive capacity (Amerah *et al.*, 2008; Svihus, 2011) as well as improving health by reducing pathogenic microbiota (Engberg *et al.*, 2004). Nir *et al.* (1994) defined a fine diet as having a geometric mean diameter (GMD) of < 574 µm and a coarse diet having a GMD > 871 µm. A coarse diet increased pepsin secretion in the proventriculus, improved physical functionality of the gizzard muscles, feed intake and body weight gain (Gabriel *et al.*, 2006). The addition of coarse particles to the diet increased gastric reflux, exposing the feed to pepsin and hydrochloric acid in the gizzard (Gabriel *et al.,* 2008; Pacheco *et al.,* 2013). With coarse grinding, retention of particles in the gizzard was longer, which may have resulted in better regulation of transit time. There are studies indicating a positive correlation between gizzard weight and ileal N digestion in broilers (Pacheco *et al.,* 2013). This may be due to an improved functionality of the gizzard, which provides more exposure of pepsin and hydrochloric acid to dietary proteins, thereby enhancing the initiation of protein digestion. It has been reported that whole wheat (coarse diet) feeding stimulated gizzard activity resulting in a high bile acid concentration in the gizzard (Svihus *et al.*, 2004). This could be associated with stimulation of pancreatic secretions due to an increase in gizzard activity (Hetland *et al.*, 2003). In contrast, diets composed of fine particles increased the digesta viscosity (Amerah *et al.,* 2007) which decreased nutrient digestibility (Yasar, 2003). It can be assumed, therefore, that increasing feed particle size may help in decreasing the amount of indigestible protein reaching the hindgut due to an improved digestion and absorption in the foregut.

Table 4 Changes (%) in gizzard weight, body weight (BW), feed conversion ratio (FCR) and duodenal villus height in relation to diet structure.

Diet treatments	Gizzard weight	BW	FCR	Villus height	Reference
Fine[1] *vs.* coarse soybean meal	+11.0	-9.8	-4.1	ND	Pacheco *et al.* (2013)
Fine[1] *vs.* ground corn and soybean meal	+10.6	+2.0	+4.9	ND	Jacobs *et al.* (2010)
Fine[1] *vs.* coarse	+34.0	+5.7	-6.0	+3.0	Amerah *et al.* (2008)
Complete ground[1] *vs.* whole wheat	+25.8	+4.3	+12.0	+6.0	Gabriel *et al.* (2008)
Ground[1] *vs.* whole wheat	+50.0	-5.0	+0.6	-2.6	Williams *et al.* (2008)
Ground[1] *vs.* whole wheat	+72.0	-1.1	-5.8	ND	Engberg *et al.* (2004)
Ground[1] *vs.* whole wheat	+23.7	+1.5	-2.0	ND	Svihus *et al.* (2004)
Complete ground[1] *vs.* whole wheat	+100.0	+14.0	0.0	ND	Gabriel *et al.* (2003)
Fine[1] *vs.* coarse	+7.2	-1.7	0.0	ND	Engberg *et al.* (2002)
Hammer mill[1] *vs.* roller mill	+8.9	+7.2	-2.9	ND	Nir *et al.* (1995)
Fine[1] *vs.* coarse corn, wheat and sorghum	+23.3	+9.9	+3.0	ND	Nir *et al.* (1994)

[1]Control diet, ND=Not determined.

PRE- AND PROBIOTICS

Pre- and probiotics may shift the balance of the gut microbiota towards an increase in the numbers of potential health-promoting bacteria such as *Lactobacilli* and

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Bifidobacteria (De Preter *et al.,* 2011). A prebiotic is defined as a food ingredient that is not hydrolysed by the animal's own digestive enzymes in the upper GIT, but positively affects the host by stimulating the growth and activity of health beneficial microbiota in the colon (Gibson *et al.*, 2004). Prebiotics are mostly carbohydrates and they can decrease proteolytic fermentation by (i) a rapid fermentation of the prebiotic substrate in the colon resulting in a lower colonic pH that reduces activity of peptides by bacterial proteases (Rastall, 2004), (ii) catabolite repression, by depressing the transcription of genes involved in amino acid catabolism in the presence of carbohydrates (Vince and Burridge, 1980), and (iii) an increased amino acid use for bacterial biosynthesis (Cummings and Bingham, 1987). The potential beneficial effects of prebiotics may include antagonism towards pathogens.

Rehman *et al.* (2008) studied the effect of inulin (1%) supplementation as a prebiotic in a basal corn-soya based diet of broilers and reported a lower caecal pH (6.4 *vs*. 7.1), lower caecal ammonia contents (13.9 *vs*. 22.3 µmol/g of digesta), lower BCFA concentrations (1.0 *vs.* 0.6 mol % of total SCFA), greater concentration of butyric acid (15.0 *vs.* 7.4 mol % of total SCFA) and numerically lower concentrations of total caecal biogenic amines (346.8 *vs.* 398.3 nmol/g of digesta) with inulin supplementation. These data suggest that inulin supplementation resulted in promoting saccharolytic instead of peptidolytic activity in the GIT as indicated by the fermentation products. These authors concluded that inulin can affect fermentation patterns, as indicated by lower caecal ammonia and a greater concentration of butyrate. Mookiah *et al.* (2014) studied the effects of different levels (5 or 10 g/kg of basal diet) of isomalto-oligosaccharides, as a prebiotic, on performance, caecal microbiota population and caecal fermentation characteristics in broilers. These authors found that total caecal VFA, acetic, propionic and butyric acid levels were increased by 43, 46, 65 and 36%, respectively in broilers fed isomalto-oligosaccharides supplemented diets compared with those fed the control diet at 42 days of age. This suggested a greater concentration of total VFA in caecal digesta of broilers fed diets supplemented with a prebiotic may be due to higher density of *Lactobacilli* and *Bifidobacteria* and lower *E. coli* and total aerobe population in the caeca. Similarly, Faber *et al.* (2012) studied the effects of oligosaccharides in corn-soybean meal based diets on immune response in *Eimeria acervulina* challenged broilers. These authors reported improved performance, and greater SCFA production in SBM-fed birds compared with SPI diet fed broilers. Caecal acetate, propionate, butyrate and total VFA concentration was increased 3.6, 1.2, 3.9, and 3.4 folds in SBM fed broilers compared with those fed the SPI diet. These authors suggested that this increased concentration of VFA may be attributed to fermentable oligosaccharides present in SBM and these oligosaccharides are the main energy source for the intestinal epithelial cells and also stimulate cell growth and improve caecal health by improving caecal weight and producing more VFA.

Probiotics are live organisms, which are beneficial to the host when regularly provided in the diet in adequate quantities (Sanders, 2008). Probiotic administration may influence the formation of SCFA. Analogue to prebiotics, probiotics may stimulate bacterial activity in the gut, resulting in an increased uptake of N, amino acids or metabolic products into the bacterial fraction (Salminen *et al.,* 1998). Probiotics may exert an indirect effect on carbohydrate or protein fermentation and enrich the population of gut microbiota with those species that preferentially ferment carbohydrates and have little proteolytic activity. Mookiah *et al.* (2014) used a multi-strain probiotic (consisting of 11 *Lactobacillus* strains) and studied the performance and caecal fermentation characteristics in broilers. These authors reported that the total caecal concentration of acetic , propionic, butyric and VFA was increased by 38, 92, 11 and 34%, respectively, in broilers fed diet supplemented with multi-strain probiotics

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compared with those fed a control diet at 42 days of age. Similarly, supplementation with *Lactobacillus acidophilus* reduced intestinal putrefactive compounds such as ammonia and biogenic amines in poultry (Gallazzi, 2009). Chen *et al.* (2012) studied the effects of probiotics (*Bacillus subtillis* and *Lactobacillus acidophilus*) supplementation in broilers. These authors reported 33.1 and 28.7% lower faecal ammonia and 49.5 and 38.6% lower H_2S, respectively in 35 days old broilers fed a probiotic supplemented diet compared with those on control and antibiotic (flavomycoin) supplemented diets. According to these authors, probiotics reduced the pathogenic microbiota in the GIT by reducing the intestinal pH, which resulted in lower faecal ammonia and H_2S emission. Based on observations in poultry, it can be assumed that prebiotic and probiotic supplementation in diets may reduce hindgut protein fermentation by decreasing the pathogenic microbial count in the caeca.

OTHER FEED ADDITIVES

Feed additives can maintain gut health through different mechanisms such as shifting gut pH, enhancement in pancreatic juice secretion, increasing nutrient intake, motivating the humoral immune response, selecting beneficial microbiota or increasing fermentation acids, and consequently reducing the invasion of pathogenic microbiota (such as *Salmonella enteritidis* and *Escherichia coli* in the host) and increasing growth rate of the intestinal mucosa (Cummings and Macfarlane, 2002). Supplementation of organic acids, especially SCFA which have specific antimicrobial activity, is a promising strategy to improve gut integrity (Adil *et al.*, 2010). Their supplementation results in less damage to epithelial cells because of a reduced production of toxic compounds as numbers of pathogenic microbiota are reduced (Antongiovanni *et al.*, 2009). Organic acids, due to their bactericidal effects, suppress protein fermenting microbiota, especially the gram negative population in broilers (Gunal *et al.,* 2006) by disrupting their energy metabolism (Ricke, 2003) and decreasing the hindgut pH. Feed ingredients, such as oligosaccharides and NSPs, affect hindgut pH by the production of VFAs (Van Der Waaij and Nord, 2000). These VFAs have a positive effect on intestinal health by providing a readily available energy source. Energy requirements of the GIT are higher compared with other body tissues. The GIT comprises 6% of the total body mass, whereas it consumes about 25% of total oxygen consumed (Britton and Krehbiel, 1993). Reduced concentrations of SCFA, especially butyrate, may lead to ulcerative colitis, reduced gut mucosal barrier function and inflammatory conditions (Wächtershäuser and Stein, 2000). Butyric acid stimulates intestinal development, *e.g.* epithelial cell proliferation and differentiation (Dalmasso *et al.*, 2008) and maintains villus height (Hu and Guo, 2007). Jang (2011) reported an 8.6% improvement in FCR in broilers supplemented with 0.2% butyric acids glycerides. Butyric acid has several functions including stimulation and production of peptides, by attaching to specific G-protein-coupled receptors, especially GPR 41 and GPR 43 (Le Poul *et al.*, 2003) in the hindgut (Tazoe *et al.*, 2008). Positive effects of some of these peptides have been reported for immunological development in health challenging situations, whereas some others have been reported to optimize gut motility (Tazoe *et al.*, 2008). It has been shown to stimulate the immune system and reduce *Salmonella spp.* colonisation in the broiler GIT (Van Immerseel *et al.*, 2005). The effects of butyric acid supplementation on broiler performance are summarised in *Table 5*. In general, the use of SCFA (such as butyric acid) in the feed of poultry is considered a possible alternative to the use of antimicrobial growth promoters (Leeson *et al.,* 2005).

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Table 5 Effects of dietary butyric acid inclusion on broiler performance and duodenal villus height.

Inclusion level range (%)	Optimal	Performance change[1] (%)			Reference
		BW	FCR	Villus height	
0.15	0.15	0	0	+15.9	Jerzsele *et al.* (2012)
2.0, 3.0	3.0	+9.3	-8.4	+21.0	Adil *et al.* (2011)
0.2	0.2	+4.6	-8.0	ND	Jang (2011)
2.0, 3.0	3.0	+6.8	-8.9	+7.5	Adil *et al.* (2010)
0.2, 0.35, 0.5, 1.0	0.2	-5.6	-4.7	ND	Antongiovanni *et al.* (2009)
0.2, 0.4, 0.6	0.4	+5.6	-8.0	+8.2	Panda *et al.* (2009)
0.2, 0.3	0.3	+8.0	-17.5	ND	Taherpour *et al.* (2009)
0.05, 0.1, 0.2	0.2	-3.0	+4.6	+10.0	Hu and Guo (2007)
0.1, 0.2	0.2	+0.6	-5.3	+9.4	Leeson *et al.* (2005)

[1]Related to optimal inclusion level, BW=Body weight, FCR=Feed conversion ratio, ND=Not determined.

Conclusions

There are no direct studies investigating the effects of gastric protein fermentation on the performance of broilers. Metabolites of protein fermentation, however, have been reported in high concentrations in the digesta collected from the ileum and caeca in broilers. Greater concentrations of biogenic amines, BCFA, H_2S, ammonia, indole, phenols, cresol and skatole in the caecal versus ileal digesta have indicated more proteolytic fermentation. Low concentrations of some of the protein fermentation products including biogenic amines are necessary for the normal gut development. The precise effects of hindgut protein fermentation on gut health and performance in broilers remain poorly understood. Further studies should elucidate the impact of protein fermentation on performance of broilers. Nutritional strategies, such as reducing dietary CP, dietary supplementation of pre- and probiotics and organic acids, or feeding diets with larger particle sizes, may increase protein digestibility and reduce undigested protein fermentation in the large intestine. Most of these nutritional interventions can potentially enhance protein digestion in the upper GIT and, therefore, less undigested protein will be available for fermentation in the hindgut.

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Table 1 Bacterial concentration in different segments of gastrointestinal tract (GIT) in broilers.

GIT	Bacterial species (log cfu/g of contents)								Reference
segment	Bacteroides	Clostridium spp.	Bifidobacterium spp.	*Enterococcus* spp.	Streptococcus spp.	Enterobacteriacea	Lactobacillus spp.	E. coli	
Crop	ND	ND	ND	4.8	ND	7.6	7.9	ND	Kročko *et al.* (2012)
	ND	BDL	ND	6.5	ND	6.7	9.0	ND	Bjerrum *et al.* (2006)
	ND	ND	ND	4.2	ND	6.6	ND	ND	Danicke *et al.* (1999)
	ND	ND	ND	ND	ND	5.8	7.6	ND	Rubio *et al.* (1998)
	10.8	8.9	7.2	3.7	8.6	10.1	8.4	ND	Takahashi *et al.* (1982)
	ND	ND	ND	ND	4.0	ND	8.7	1.7	Smith (1965)
Gizzard	ND	ND	ND	5.1	ND	ND	6.2	4.4	Bjerrum *et al.* (2005)
	ND	ND	ND	5.1	ND	ND	6.8	4.4	Engberg *et al.* (2004)
	ND	ND	ND	ND	ND	ND	6.4	3.4	Chen (2003)
	ND	ND	ND	4.3	ND	ND	6.6	4.3	Engberg *et al.* (2002)
	ND	ND	ND	5.0	ND	ND	7.5	4.8	Engberg *et al.* (2000)
	ND	ND	ND	ND	3.7	ND	7.3	ND	Smith (1965)
Jejunum	ND	4.8	ND	5.7	ND	ND	7.5	5.7	Engberg *et al.* (2004)
	ND	ND	ND	5.8	7.4	ND	8.0	5.5	Engberg *et al.* (2002)
	ND	ND	ND	5.9	ND	ND	8.1	6.3	Engberg *et al.* (2000)
	6.5	6.5	ND	ND	5.3	ND	6.5	5.7	Smits *et al.* (1998)
Ileum	ND	ND	ND	3.7	ND	5.9	7.4	ND	Kročko *et al.* (2012)
	ND	BDL	ND	6.5	ND	ND	9.0	6.7	Bjerrum *et al.* (2006)
	ND	6.4	ND	7.1	ND	ND	8.5	7.4	Engberg *et al.* (2004)
	ND	5.9	7.3	ND	ND	ND	7.5	6.9	Xia *et al.* (2004)
	ND	ND	7.2	ND	ND	ND	7.5	7.0	Xu *et al.* (2003)
	7.7	7.7	ND	ND	6.6	ND	7.7	7.7	Smits *et al.* (1998)
Caeca	9.9	10.1	9.7	ND	ND	ND	9.4	ND	Jozefiak *et al.* (2010)
	ND	3.7	6.9	ND	ND	ND	6.4	3.6	Tiihonen *et al.* (2010)
	BDL	ND	ND	ND	6.7	ND	9.2	6.7	Bjerrum *et al.* (2006)
	11.2	4.3	8.4	ND	ND	ND	10.1	6.9	Cao *et al.* (2005)
	ND	6.5	ND	ND	7.1	ND	8.8	8.7	Engberg *et al.* (2004)
	ND	6.6	8.2	ND	ND	ND	8.4	7.5	Xia *et al.* (2004)
	6.7	ND	8.6	ND	7.4	ND	7.6	7.0	Guo *et al.* (2003)
	8.7	1.7	6.9	ND	6.7	ND	8.7	5.6	Smith (1965)

cfu=colony forming unit, ND=Not determined, BDL=below detection level.

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Table 2 Difference between ileal and excreta apparent digestibility (%) of different amino acids from different feed stuffs in broilers.

Ingredient	Essential amino acids									Reference
	Thr	Val	Met	Iso	Leu	Phe	His	Lys	Arg	
Blood meal	-14.2*	+0.4	+2.1	0	-0.1	+3.8	+2.2	-3.2	-3.7	1
Canola meal	-1.0	+2.0	+1.0	+2.0	+1.0	+2.0	+1.0	+1.0	-2.0	2
Cotton seed meal	-1.0	+3.0	+6.0*	+3.0*	+3.0*	+2.0	+1.0	+6.0*	-1.0	2
Feather meal	-24.0*	-14.0	-12.0	-12.0	-14.0	-8.0	-12.0	-20.0*	-16.0*	2
Fish meal	-7.6*	-0.6	+5.4	-0.3	-5.4	-0.3	-0.3	-1.3	-1.3	1
	-7.0*	+3.0*	0	+4.0*	0	0	-4.0	+1.0	-2.0*	2
Maize	-7.0	+6.0*	-1.0	+8.0*	+1.0	+3.0	0	-1.0	+1.0	2
Meat meal	-19.0 - -6.0	-11.0 - 0	-11.0 - -3.0	-10.0 - -2.0	-11.0 - 0	-3.0 - - 1.0	-6.0 - +3.0	-16.0 - -1.0	-13.0 - -3.0	2
Meat and bone meal	-16.7*	+5.1	+4.9	-7.3*	-8.6*	+2.2	-3.6	-8.0*	+0.7	1
	-12.0*	-5.0	-6.0*	-5.0	-6.0	+3.0	+3.0	-14.0*	-6.0	2
Sorghum	-8.7*	-0.6	-0.8	-5.5	-2.3	+1.2	+2.8	-5.5	+3.9	2
	+3.0	+7.0	+1.0	+8.0	+3.0	+4.0	+12.0*	+10.0	+4.0	2
Soybean meal	-7.7*	-4.4	+2.6	-0.6	-3.9	+1.3	-3.1	-1.0	-1.9	1
	-5.0 - -2.0*	0 - +5.0*	+1.0 - +9.0	0 - +5.0*	-1.0 - +1.0*	0 - +1.0*	-	-3 - +2	-2.0 - -4.0*	2
Wheat	- 19.6*	-11.3	-1.6*	-18.2*	-20.4*	-6.9*	-16.0*	-9.5*	-20.3*	1
	+ 20.0*	+15.0*	+7.0*	+14.0	+11.0	+10.0	+18.0	+17.0*	+7.0*	2

	Non essential amino acids						
	Asp	Ser	Glu	Ala	Pro	Tyr	
Blood meal	-16.9*	-0.5	-10.3*	+7.1*	-3.9	+3.3	1
Canola meal	-2.0	-3.0	+1.0	+4.0	ND	0	2
Cotton seed meal	-1.0	-2.0	+2.0*	+8.0*	ND	+1.0	2
Feather meal	-37.0*	-21.0*	-18.0	-12.0	ND	-13.0	2
Fish meal	-1.3	-4.8	-6.2	+1.3	+0.2	+1.2	1
	-9.0*	-9.0*	0	+4.0	ND	+2.0	2
Maize	0	-5.0	+2.0*	+5.0*	ND	-1.0	2
Meat meal	-30.0 - -18.0	-21.0 - -10.0	-14.0 - - 4.0	-13.0 - 0	ND	-8.0 - -8.0	2
Meat and bone meal	-17.1*	-7.3*	-10.0*	-13.0*	-4.9	-7.9*	1
	-21.0*	-13.0*	-7.0	-7.0	ND	-2.0	2
Sorghum	-2.9	-8.8*	-0.1	-0.2	-0.1	-2.1	1

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Table 2 Continued

Ingredient	Essential amino acids									Reference
	Thr	Val	Met	Iso	Leu	Phe	His	Lys	Arg	
	+4.0		0	+4.0		+8.0	ND		-4.0	2
Soybean meal	-2.5		-5.8	-6.6		-4.8	-8.0*		-4.5	1
	-5* - -4.0*		-10 - -4.0*	0 - +2		+2 - +9.0*	ND		-1 - +1.0	2
Wheat	-31.3*		-6.5*	-4.3		-9.9*	-4.3		-15.7*	1
	+18.0		+11.0*	+6.0*		+25.0*	ND		+5.0*	2

Values with an * indicate significant (P<0.05) differences between ileal and excreta apparent digestibilities, ND=Not determined, Difference=Ileal - excreta digestibility. [1]Kadim *et al.* (2002). [2]Ravindran *et al.* (1999).