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Endogenous flow of amino acids in the avian ileum as influenced by increasing dietary peptide concentrations

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The aim of the present study was to establish whether feeding broiler chickens with diets containing increasing dietary peptide concentrations would cause increases in ileal endogenous amino acid flow. The flow of N and most amino acids increased quadratically ($P<0{\cdot}05$ to $0{\cdot}001$) with increasing dietary concentrations of peptides. The exceptions were the flow of threonine, serine, glycine, tyrosine and cystine, which increased linearly ($P<0{\cdot}001$) with dietary peptide levels. Another notable exception to the general trend was the flow of proline, which was significantly higher ($P<0{\cdot}01$) in birds fed the protein-free diet. The amino acid profile of endogenous protein, expressed as proportion of crude protein, indicated that the ratios of threonine, glutamic acid, proline, glycine, leucine, histidine, arginine and cystine were influenced ($P<0{\cdot}05$) with increasing dietary peptide concentrations. In general, compared with the protein-free diet, the ratios of threonine and arginine in endogenous protein were lower ($P<0{\cdot}05$) and those of glutamic acid, glycine and histidine were greater ($P<0{\cdot}05$) in diets with high concentrations of peptides. The ratio of proline was found to decrease ($P<0{\cdot}05$) with increasing dietary peptide concentrations. These changes in the amino acid profile of endogenous protein are probably reflective of changes in the output of one or more of the components of endogenous protein. Overall, the present results demonstrated that increasing dietary peptide concentrations increased the flow of endogenous amino acid flow at the terminal ileum of broiler chickens in a dose-dependent manner and also caused changes in the composition of endogenous protein. The observed changes in endogenous amino flow will influence the maintenance requirements for amino acids and also have implications for the calculation of true digestibility coefficient of feedstuffs.

Ileal endogenous amino acid flow: Enzyme-hydrolysed casein: Chickens

During the digestion and absorption of ingested feed, significant amounts of endogenous N enter the gastrointestinal tract at various segments. These inevitable secretions containing N predominantly originate from various digestive secretions, mucoproteins and desquamated epithelial cells lining the gut[1,2]. A proportion of these secretions escapes digestion and re-absorption and amino acids (AA)[2] from endogenously secreted proteins that reach the terminal ileum are lost to the animal. Reliable estimates of these losses of N and AA are necessary for the calculation of true digestibility values of feed ingredients and for the determination of AA requirements by the factorial method. The true ileal AA digestibility of an ingredient is a better measure than apparent digestibility because it represents the AA actually released from dietary protein and absorbed by the animal, and yields more precise estimates of the amount of AA provided in a mixed diet.

In poultry, the endogenous flow of AA has traditionally been determined by the measurement of AA excretion at the excreta level in fasted birds or in birds fed on protein-free diets[3]. Both these conditions are physiologically abnormal and could influence body protein catabolism and digestive enzyme secretions in the gut. Furthermore, AA that disappear post-ileum are not considered beneficial to chickens because their disappearance is due to hindgut microbial action and not absorption[4]. Thus AA digestibility must be measured at the terminal ileum rather than in the excreta[5] and it is the endogenous AA flow at the ileal level that should be considered as true losses to the animal.

An approach that overcomes the limitations of the traditional methods and that can be used for routine determination of ileal endogenous AA flow under conditions of peptide alimentation was proposed by Moughan *et al.*[6]. In this method, the animal is fed a purified diet containing enzyme-hydrolysed casein (EHC) as the sole N source. The hydrolysed casein consists of small peptides and free AA (molecular weight <5000 Da), which act to maintain physiologically normal levels of endogenous N flow throughout the intestinal tract. Ileal digesta are collected, centrifuged and the supernatant fraction is ultrafiltered (exclusion limit, molecular weight 10 000 Da). The precipitate from the centrifugation step and the retentate from the ultrafiltration step ($>10\,000$ Da) are assumed to represent the endogenous component of the digesta. This method has been used to measure ileal AA losses in pigs[7–10], in rats[11] and in chickens[12,13].

Studies comparing protein-free and EHC diets have shown that the presence of dietary peptides results in marked increases in the endogenous flow of N and AA[1,13–15].

Abbreviations: AA, amino acid; EHC, enzyme-hydrolysed casein.

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The effect of only a single inclusion level of EHC was tested in these studies and it is not known whether this is a one-off effect or whether the inclusion of graded levels of dietary peptides would cause gradual increases in ileal endogenous flows of N and AA. A study by Hodgkinson *et al.* [10], with pigs, showed that ileal endogenous AA flow increased when dietary EHC concentrations were increased from 0 to 200 g/kg. In contrast, using guanidinated soyabean meal, Zhang *et al.* [16] found that increasing the dietary protein content from 100 to 250 g/kg had no effect on the ileal endogenous flow of lysine in growing pigs. The aim of the present study was to examine the effects of graded dietary concentrations of EHC on the endogenous AA flow in the avian ileum.

Materials and methods

Diets

Six diets were formulated. These included a basal diet and five test diets that contained 0 (protein-free diet), 50, 100, 150 and 200 g EHC/kg (Table 1). In the latter four diets, EHC served as the sole source of N. Titanium oxide (3 g/kg) was included in all test diets as an inert digesta marker for the calculation of endogenous AA flows. The basal diet was similar to the EHC diet except that casein was used in place of EHC and that no titanium oxide was included.

Birds

Male broiler (Ross 308) chicks were obtained at 1 d of age from a commercial hatchery and raised in floor pens as per standard commercial practice. The birds were fed a commercial starter diet, in mash form, based on wheat, soya and meat meal (12·6 MJ metabolisable energy/kg, 236 g crude protein/kg, 96 g Ca/kg and 48 g available P/kg) till 21 d of age. On day 21 post-hatching, the birds were individually weighed and eighty birds (average weight 880 g) were selected and assigned to twenty cages of four birds each. The five dietary treatments were then randomly assigned to four cages each.

The cages were housed in an environmentally controlled room with 24 h fluorescent lighting. Room temperature was maintained at 24 ± 2°C during the assay. The experimental procedures were approved by the Animal Ethics Committee of Massey University.

Conduct of the trial

From age 21 d to 28 d, the birds were first trained to consume a mash diet, which was made by grinding commercial starter pellets in a hammer-mill to pass through a 3 mm screen. After 1 week on the mash diet, the basal diet was gradually introduced. The aim of using the casein-based basal diet was to enable the birds to get adjusted to the changeover to purified diets. The basal diet was withdrawn after 4 d and the test diet containing EHC was introduced on day 32 post-hatch. The diets were offered *ad libitum* and water was available at all times.

Digesta collection and processing

After 4 d on the EHC diet, the birds were euthanised by an intracardial injection of sodium pentabarbitone, and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocaecal junction. Samples from birds within a pen were pooled, homogenised, frozen immediately after collection and lyophilised[17].

The following procedure[15] was used to separate the endogenous protein fraction in the ileal digesta of birds fed the EHC diets. The lyophilised samples were re-suspended in deionised water and acidified to pH 3·5 with 9 M-H_2SO_4. The samples were stored overnight at 4°C and then centrifuged

Table 1. Composition (g/kg) of the basal diet, protein-free diet and the test diets containing graded levels of enzyme-hydrolysed casein (EHC)

	Diet					
	Basal*	Protein-free	50 g EHC/kg	100 g EHC/kg	150 g EHC/kg	200 g EHC/kg
Casein	180	–	–	–	–	–
EHC†	–	–	50	100	150	200
Dextrose	670	847	797	747	697	647
Vegetable oil	50	50	50	50	50	50
Cellulose	35	35	35	35	35	35
Dicalcium phosphate	24	24	24	24	24	24
Sodium bicarbonate	20	20	20	20	20	20
K_2HPO_4	12	12	12	12	12	12
Salt	4	4	4	4	4	4
Titanium oxide	–	3	3	3	3	3
MgO	2	2	2	2	2	2
Mineral premix‡	2·5	2·5	2·5	2·5	2·5	2·5
Vitamin premix§	0·5	0·5	0·5	0·5	0·5	0·5

* The basal diet was fed to all birds for 4 d before the introduction of the EHC diet.
† New Zealand Pharmaceuticals Ltd (Palmerston North, New Zealand). The molecular-weight distribution was determined using gel permeation chromatography. All samples were less than 5000 Da in size, with 99 % less than 3000 Da.
‡ Supplied (per kg diet): Mn, 125 mg; Zn, 60 mg; Cu, 3 mg; Mo, 0·5 mg; Co, 0·3 mg; I, 1 mg; Fe, 25 mg; Se, 200 μg; choline chloride, 638 mg.
§ Supplied (per kg diet): *trans*-retinol, 3·33 mg; cholecalciferol, 60 μg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamine, 3·0 mg; riboflavin, 12 mg; calcium pantothenate, 12·8 mg; niacin, 35 mg; pyridoxine, 10 mg; folic acid, 5·2 mg; cyanocobalamin, 0·017 mg; biotin, 0·2 mg; antioxidant, 100 mg.

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at 1450 ***g*** for 45 min at 0°C. The supernatant fraction was decanted off and retained. The precipitate was washed with 10 ml deionised water and centrifuged at 1450 ***g*** for 30 min at 0°C. The second supernatant fraction was added to the first and the precipitate was stored at −20°C. The combined supernatant fractions were ultrafiltered using a Centriprep-10 ultrafiltering device (molecular weight cut-off filter, 10 000 Da; Amicon Inc., Beverly, MA, USA) according to the manufacturer's instructions. The precipitate from the centrifugation step was added to the retentate (> 10 000 Da) from the ultrafiltration step, and the material was lyophilised.

Diet and dried ileal digesta samples were then ground to pass through a 0·5 mm sieve and stored in airtight containers at −4°C for chemical analyses.

Chemical analyses

The diets, digesta samples from birds fed the protein-free diet and ultrafiltered ileal digesta samples from birds fed the EHC diets were analysed for DM, N, AA and titanium as described below.

DM determination was carried out according to standard procedures[18]. Total N was determined following Kjeldahl digestion by colorimetric auto-analyser[19]. AA were determined by hydrolysing the samples with HCl (containing phenol) for 24 h at 110 ± 2°C in glass tubes sealed under vacuum. AA were detected on a Waters ion exchange HPLC system, and the chromatograms were integrated using dedicated software (Millenium 32; Waters, Millipore, Milford, MA, USA) with the AA identified and quantified using a standard AA solution (Pierce, Rockford, IL, USA). Cystine and methionine were analysed as cysteic acid and methionine sulfone by oxidation with performic acid for 16 h at 0°C and neutralisation with hydrobromic acid before hydrolysis. Tryptophan was not determined. Titanium content was measured on a UV spectrophotometer following the method of Short *et al.* [20].

Calculations

The flow of N and individual AA at the terminal ileum was calculated, as mg lost per kg ingested feed DM, using the following formula and these values were considered to be the estimate for endogenous flow[21].

$$\text{Endogenous N or AA flow (mg/kg DM intake)} = \text{N or AA concentration in ileal digesta (mg/kg)} \times (\text{diet titanium (mg/kg)}/\text{ileal digesta titanium (mg/kg)}).$$

The AA profile of endogenous protein (N × 6·25) was calculated by expressing each AA as a percentage of endogenous crude protein.

Statistical analysis

The data were tested for homogeneity of variance using Bartlett's test[22]. The statistical analyses used the general linear models procedure of SAS (SAS Institute, Inc., Cary, NC, USA)[23] with pen means as the experimental unit. Where appropriate, significant differences between means were separated using the least significance difference test. Linear and quadratic effects of graded dietary levels of EHC (0, 50, 100, 150 and 200 g/kg) on endogenous flow of N and AA were tested with orthogonal polynomials[23].

Results

During the 4 d assay period, feed intake was similar ($P > 0{\cdot}05$) between the dietary treatments. Average daily feed intake of birds fed diets containing 0, 50, 100, 150 and 200 g EHC/kg were 65, 70, 78, 74 and 68 (SEM 4·5) g/bird, respectively.

The determined crude protein and AA contents of the test diets are shown in Table 2. The ileal endogenous flows of N and AA in birds fed diets containing graded concentrations of EHC are summarised in Table 3. The flows of N and most AA

Table 2. Determined crude protein and amino acid concentrations (g/kg DM) of the protein-free diet and the test diets containing graded levels of enzyme-hydrolysed casein (EHC)

	Diet				
	Protein-free	50 g EHC/kg	100 g EHC/kg	150 g EHC/kg	200 g EHC/kg
Crude protein (N × 6·25)	3·88	48·2	92·6	145·5	198·4
Aspartic acid	0·17	3·55	7·56	10·70	14·74
Threonine	0·06	1·79	3·44	5·32	7·21
Serine	0·07	2·34	4·36	7·16	9·20
Glutamic acid	0·26	10·40	19·90	31·35	42·10
Proline	0·00	4·89	9·25	14·69	19·62
Glycine	0·12	0·94	1·64	2·58	3·45
Alanine	0·12	1·42	2·45	3·91	5·34
Valine	0·10	2·92	5·56	8·63	11·32
Isoleucine	0·12	2·36	4·59	6·99	9·40
Leucine	0·17	4·28	8·35	12·78	17·45
Tyrosine	0·07	0·98	1·85	3·02	4·00
Phenylalanine	0·07	2·19	4·34	7·22	9·47
Histidine	0·14	1·42	2·58	4·16	5·45
Lysine	0·16	3·71	7·04	10·66	14·52
Arginine	0·08	1·37	2·92	4·88	6·30
Methionine	0·00	0·84	0·87	0·96	1·13
Cystine	0·00	1·59	2·91	4·89	5·92

Table 3. Ileal endogenous flows (mg/kg DM intake) in 5-week-old broiler chickens fed diets containing graded levels of enzyme-hydrolysed casein (EHC) (Mean values for four replicates of five birds each with their pooled standard errors)

	Diet						Significance	
	Protein-free diet	50 g EHC/kg	100 g EHC/kg	150 g EHC/kg	200 g EHC/kg	SEM	Linear	Quadratic
N	1232	1311	1733	2096	2722	106·3	***	***
Aspartic acid	549	674	887	1050	1345	45·5	***	NS
Threonine	527	583	776	886	1037	59·9	***	NS
Serine	414	522	611	727	972	55·9	***	NS
Glutamic acid	689	903	1170	1941	2485	84·0	***	NS
Proline	1070	561	595	590	706	44·8	*	*
Glycine	477	698	868	1011	1461	65·1	***	NS
Alanine	292	351	327	484	606	31·8	***	**
Valine	282	346	485	614	831	39·7	***	**
Isoleucine	252	246	342	422	573	30·5	***	**
Leucine	363	350	619	702	1002	58·0	***	**
Tyrosine	206	192	285	326	395	20·8	***	*
Phenylalanine	195	231	238	320	401	20·4	***	**
Histidine	133	123	243	298	379	18·9	***	**
Lysine	225	251	343	464	579	33·8	***	**
Arginine	238	245	281	321	404	28·1	***	**
Methionine	89	109	120	151	202	16·2	***	**
Cystine	201	235	263	295	355	13·4	***	NS
Sum of amino acids	6201	6619	8452	10 603	13 753	310·1	***	**
Amino acid-N	922	968	1220	1540	1990	44·5	***	**
Amino acid-N, as % total N	75·0	73·9	70·6	73·6	73·1	1·61	NS	NS

*$P<0·05$, **$P<0·01$, ***$P<0·001$.

increased quadratically ($P<0·05$ to $0·001$) with increasing dietary levels of EHC. The exceptions were the flows of aspartic acid, threonine, serine, glutamic acid, glycine and cystine, which increased linearly ($P<0·001$) with dietary EHC levels. The flow of proline was another exception and this was markedly higher in birds fed the protein-free diet and unaffected when dietary EHC levels were increased from 50 to 200 g/kg. In general, the flow of N and individual AA (except that of proline) determined for the protein-free diet and the 50 g EHC/kg diet were similar, but the flow increased when the EHC was included at or above 100 g/kg. The ileal flow of N and total AA in birds fed the 200 g EHC/kg diet were 2·2-fold greater than those determined for the protein-free diet, except for proline. The flow of individual AA in birds fed the 200 g/kg diet ranged from 1·8 (cystine) to 3·6 (glutamic acid) times higher than those fed the protein-free diet.

Glutamic acid was the most abundant AA in the endogenous flow (Tables 3 and 4). Other AA that were present in relatively high concentrations were glycine, threonine, aspartic acid, serine, valine and isoleucine. Concentration of methionine was the lowest.

The AA profile of endogenous protein, expressed as a proportion of crude protein, indicates that threonine, glutamic acid, proline, glycine, leucine, histidine, arginine and cystine ratios were influenced ($P<0·05$) by dietary treatments (Table 4). In general, compared with the protein-free diet, the proportions of threonine and arginine in endogenous protein were lower ($P<0·05$) and those of glutamic acid, glycine and histidine were greater ($P<0·05$) in diets with 200 g EHC/kg. Increasing the dietary EHC concentration from 50 to 200 g/kg had no effect ($P>0·05$) on the ratio of most AA in the endogenous protein. The exceptions were glutamic acid, proline, histidine and cystine. The ratios of proline and cystine were found to decrease ($P<0·05$) with increasing dietary EHC concentrations, while those of glutamic acid and histidine were greater ($P<0·05$) at higher inclusions of EHC.

Discussion

The aim of the present study was to investigate whether increasing dietary concentration of peptides will cause increased flow of endogenous protein and AA in the avian ileum. The results showed that the endogenous flow of the N and total AA were found to be similar between the protein-free diet and the 50 g EHC/kg diet, but increased when the dietary EHC was included at or above 100 g/kg. Compared with the protein-free diet, the inclusion of 50, 100, 150 and 200 g EHC/kg increased the mean endogenous N flow by 6·4, 40·7, 70·1 and 120·9 %, respectively. The corresponding increases in the flow of total AA were found to be 6·7, 36·3, 71·0 and 121·8 %, respectively.

Constant endogenous AA values are commonly employed in corrections in the conversion of apparent digestibility coefficients to true digestibility coefficients, as it is generally assumed that dietary protein concentration has no effect of endogenous AA flow at the terminal ileum. The present results show that such an approach to calculate true AA digestibility coefficients of feedstuffs or diets with varying protein concentrations is not valid and that changes in endogenous AA flow with dietary protein concentrations need to be considered in true digestibility calculations.

It appeared that an EHC concentration of 50 g/kg was insufficient to have a significant effect on the ileal endogenous AA flow. In general, the endogenous flow of AA, with the exception of proline, followed the same pattern. The response of individual AA to increasing peptide concentrations, however, was varied. Compared with the flow in birds fed the 50 g EHC/kg diet, the increments in those fed the 200 g EHC/kg diet were

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Table 4. Amino acid composition of endogenous protein (g/kg crude protein) in broilers fed the protein-free diet and test diets containing graded levels of enzyme-hydrolysed casein (EHC)

(Mean values for four replicates of five birds each with their pooled standard errors)

	Diet					
	Protein-free diet	50 g EHC/kg	100 g EHC/kg	150 g EHC/kg	200 g EHC/kg	SEM
Aspartic acid	71·7	82·3	82·0	80·3	79·0	4·8
Threonine	68·8^{a,b}	71·1^{a}	71·9^{a}	67·5^{a,b}	60·8^{b}	3·3
Serine	53·8	63·7	56·4	55·2	57·0	4·3
Glutamic acid	90·0^{b}	110·2^{b}	108·2^{b}	148·4^{a}	146·1^{a}	6·5
Proline	139·6^{a}	68·6^{b}	55·2^{b,c}	45·2^{c}	41·5^{c}	7·9
Glycine	61·4^{b}	85·2^{a}	80·5^{a}	77·4^{a,b}	85·8^{a}	5·8
Alanine	37·7	42·9	31·3	37·0	35·8	3·9
Valine	36·9	42·2	44·8	46·9	48·8	4·5
Isoleucine	32·4	30·0	31·7	32·3	33·7	2·9
Leucine	46·9^{a,b}	42·7^{b}	56·5^{a}	53·7^{a,b}	58·8^{a}	3·5
Tyrosine	26·5	23·4	26·4	25·0	23·2	2·1
Phenylalanine	25·5	28·2	21·9	24·5	23·6	2·4
Histidine	17·3^{b}	15·1^{b}	22·6^{a}	22·7^{a}	22·3^{a}	1·5
Lysine	29·1	30·7	31·8	35·4	34·1	3·5
Arginine	30·9^{a}	30·0^{a,b}	25·8^{a,b}	24·6^{b}	23·7^{b}	2·1
Methionine	11·6	13·4	11·1	15·6	11·9	1·9
Cystine	26·4^{a,b}	28·6^{a}	24·5^{a,b}	22·5^{a,b}	20·9^{b}	2·4

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

highest for glutamic acid, leucine and histidine (175 to 206 %) and moderate for aspartic acid, threonine, serine, glycine, alanine, valine, isoleucine, tyrosine and phenylalanine (73 to 140 %). The increments were lowest for cystine (51 %) and proline (26 %).

The higher ileal endogenous AA flows in birds fed the EHC diets compared with those in birds fed the protein-free diet were consistent with previously published data in rats[11,14], pigs[10,15] and chickens[13]. The values determined for ileal losses in N and AA determined for the protein-free diet and 200 g EHC/kg diet were within the ranges reported in the literature for growing chickens[12,13,24,25].

The reasons for increased endogenous AA losses with increasing protein or peptide concentrations are not fully understood, but clearly these increases are related to increased output of one or more of the components of endogenous protein. Several possibilities may be proposed. First, increasing concentrations of dietary peptides or protein in the intestinal digesta may stimulate the secretion of endogenous enzymes. Studies with pigs have shown the increasing dietary protein levels to increase pancreatic[26] and intestinal secretions[27]. Second, dietary protein or peptides may increase the secretion of mucin. It is also possible that the enhanced production of endogenous digestive enzymes may increase the hydrolysis of the mucus layer, releasing mucin into the digesta. Mariscal-Landing *et al.* [28] reported that the ileal hexosamine excretion in pigs fed diets containing isolated soya protein increased when dietary crude protein concentration increased above 55 g/kg, suggesting a greater recovery of mucin at the terminal ileum. Another possibility is that, in addition to the direct protein effect, the EHC may contain bioactive peptides which may have specific effects in modifying the secretion of components of endogenous protein, including digestive enzymes and mucin. Partial hydrolysates of proteins have been shown to be potent stimulants of gastric acid and pancreatic secretions[29,30]. Of particular interest are the findings of Claustre *et al.* [31] that enzymic hydrolysates of casein induced a strong mucin secretion in rat jejunum and this was attributed to the presence of an opioid peptide, β-casomorphin-7. In their study, mucin secretion was unaffected by native casein. Furthermore, given that increasing the dietary protein content from 100 to 250 g/kg in diets based on guanidinated soyabean meal had no effect on ileal endogenous flow of lysine in pigs[16], it is speculated that part of the increases in ileal endogenous AA flow with increasing EHC levels may be related to the presence of specific bioactive peptides in the casein hydrolysate. In the present study, AA that are found in high concentrations in pancreatic and intestinal secretions and mucin contributed in greater proportions to endogenous AA flow as dietary peptide concentrations increased, lending support to these possibilities. Pancreatic secretions are reported to have relatively high concentrations of branched-chain AA, glycine, aspartate and glutamate[32,33], whereas biliary secretions are known to be rich in glycine[34]. The predominance of threonine and serine in intestinal mucins is also known[35]. In the present study, increasing dietary peptide concentrations resulted in higher histidine flows. The reasons for this finding are not clear, since none of these individual sources are known to contain high concentrations of this AA.

It is noteworthy, however, that the flow of endogenous AA determined at the terminal ileum represents the proportion of endogenous secretions that are not digested and/or reabsorbed[1]. The implication is that any factor that affects the reabsorption of AA of endogenous origin will also influence the estimates of endogenous AA flow. It is possible that the digestion and/or reabsorption of endogenous proteins may be down-regulated at higher peptide concentrations and this may have contributed, at least in part, to the higher flows determined at higher inclusion levels of EHC. For example, it has been suggested that the degradation of digestive enzymes may be lower in the presence of protein[36], with dietary proteins protecting digestive enzymes from hydrolysis by being preferentially used as substrates.

In the present study, an unusually higher flow of proline was determined in birds fed protein-free diets and proline dominated the AA profile of endogenous protein in the ileal digesta of these birds. Higher losses of proline have been previously reported in pigs fed protein-free diets[10,37] and this finding was attributed to disturbances in protein metabolism. The present results lend support to this suggestion, as the ileal flow of proline was markedly lowered when peptides were included in the diet. It is likely that the disturbed protein metabolism may affect the losses of other AA as well, but these effects were not evident possibly because the absorption is still positive.

In agreement with previous data from chickens[12,13] and pigs[38,39], glutamic acid, aspartic acid, threonine and glycine dominated the AA profile of endogenous protein in the ileal digesta. The impact of dietary protein or peptide concentrations on the composition of endogenous protein has not been previously reported. The ratios of most AA in the endogenous protein were unaffected when dietary EHC concentrations were increased from 50 to 200 g/kg. The exceptions were the proportions of proline and cystine which were found to decrease with increasing dietary EHC concentrations, while those of glutamic acid and histidine were greater at higher inclusions of EHC. While the effect on proline ratio is probably related to peptide alimentation, those on glutamic acid and cystine may be explained by changes in relative proportions of individual sources that contribute to the endogenous protein.

It is generally assumed that the AA composition of endogenous protein is reasonably constant[38,39]. Despite some changes in the AA profile of endogenous protein with increasing dietary peptide concentrations from 50 to 200 g EHC/kg, the present data are in general agreement with this assumption. A recent study by Cowieson & Ravindran[40], however, found that the profile of endogenous protein was altered by the dietary addition of phytic acid. These findings suggest the need for further evaluation of the effect of dietary constituents on the AA composition of endogenous proteins.

When the EHC method is employed to determine ileal endogenous AA losses, the assumption is made that there are only small amounts of endogenous peptides in ileal digesta that are smaller than 10 000 Da in size. This assumption, however, has not been validated. It is expected that the concentration of these low-molecular-weight peptides at the terminal ileum will be low, but it is difficult to accurately quantify the amounts of these molecules and it is likely that such losses may lead to some underestimation of ileal endogenous AA flow. If this is valid, then it follows that such losses may have been relatively greater in animals fed diets containing higher peptide concentrations.

From a practical point of view, accurate estimates of endogenous AA values need to be obtained to improve the precision of true digestibility calculations and feed formulations. It is generally assumed that dietary protein intake has no effect on the ileal endogenous AA losses and the common practice is to use a constant value for endogenous AA flow in the calculations of true digestibility of feed ingredients, irrespective of their protein concentrations[41]. The present results suggest that the use of one set of values for endogenous correction of feedstuffs with varying protein concentrations is not valid. It appears therefore that, for the determination of true digestibility coefficients of feedstuffs, different endogenous corrections may have to be used depending on the protein concentrations of the assay diets, which typically range from 80 g/kg for cereals to 180 g/kg for protein sources[42].

In summary, the present data established that increasing dietary peptide concentrations have a dose-dependent effect on ileal endogenous AA flow in poultry. Such quantitative changes in endogenous AA flow will influence the maintenance requirements for AA and these changes must be taken into account in diet formulations. The increased endogenous AA losses with increasing dietary peptide concentrations may be explained by increased output of one or more of the components of endogenous protein. In this context, studies to investigate the effect of increasing dietary protein concentrations using guanidinated forms of purified proteins, such as casein, soya protein and wheat gluten, will be of interest. Further studies are also warranted to understand the mechanisms underlying the observed changes with dietary peptide concentrations.

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