Endogenous amino acid loss in poultry with a special emphasis on the contribution of bacterial biomass.

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Introduction

Modern poultry genotypes are fantastically efficient converters of plant-based nutrients into saleable high-quality animal protein. However, in order to support optimal net income of poultry operations it is imperative that these modern breeds are provided with diets of consistent and high nutritional value. In the case of amino acids these are usually considered on an apparent or standardized digestible basis (the difference between the two terms recognizes the flow of basal endogenous protein) (Ravindran et al., 2017). However, although some allowances are made in diet formulation for inevitable amino acid losses from the host animal, the source of these losses is often obscure. It is therefore the purpose of this article to briefly describe endogenous amino acid loss in poultry and to offer some recommendations to minimize and accommodate this through various nutritional means.

Terminology

Endogenous protein loss is defined as any protein, peptide or amino acid that is not of dietary origin that exits the terminal ileum (Ravindran & Hendriks, 2004). These endogenous protein losses can be sub-divided into either basal (inevitable losses that are unrelated to dietary characteristics) or specific (diet dependent) flow (Cowieson et al. 2009). Basal endogenous losses are assumed to be inevitable and associated with inherent metabolic processes that are independent of the diet.

Experimentally, we measure these losses by feeding diets that are devoid of nitrogen (Ravindran et al., 2004; Adeola et al., 2016) although alternative methods exist. Specific endogenous losses are more difficult to quantify as these vary with the chemical and mechanical properties of the diet e.g. concentration of protein, presence of various anti-nutrients etc. (Ravindran, 2016).

We use the terms apparent, standardized and true in reference to amino acid digestibility. Apparent is applied when ileal amino acid digestibility in measured without simultaneous assessment of endogenous amino acid flow. Standardized refers to a nitrogen-free diet to estimate basal endogenous flow, and used to correct apparent digestibility values. Finally, when all sources of endogenous protein in the ileum are quantified (basal and specific) the 'true' digestibility of dietary protein can be determined.

Source and Significance

The input of endogenous protein into the gut during the digestive process is considerable and estimates vary from 1-4g of endogenous protein per gram of ingested protein (Ravindran, 2016). These endogenous proteins vary in amino acid composition, origin and in their recalcitrance to autolytic recovery. However, major sources of endogenous protein in the intestine of poultry include mucin, bile, sloughed epithelial cells and digestive enzymes (Moughan et al., 2014; Ravindran, 2016). As mentioned

above, estimates regarding the quantity of these endogenous losses vary (depending on methodology, animal age etc) but values of around 10-15 g/kg of dry matter intake for basal losses would cover most published examples (Ravindran, 2016).

The amino acid composition of some of these major sources of endogenous protein can be found in Table 1. Dominant amino acids in endogenous proteins are Gly, Thr, Glu, Asp, Ser, Val, Leu and Cys. Importantly, these endogenous secretions are partially recovered before they leave the terminal ileum and estimates of this range from 75-90% (Souffrant et al., 1993; Forstner & Forstner, 1994). Axiomatically then a substantial portion of these endogenous secretions exit the ileum and as a significant metabolic cost to the animal, not only in terms of amino acids *per se* but also digestible and net energy. For example, Boisen & Verstegen (2000) present the gross energy content of various amino acids and this ranges from 2892 kcal/kg for Asp to 6740 kcal/kg for Phe (Met, Lys and Thr are 4446, 5617 and 4111 kcal/kg respectively). Quantitative or qualitative changes to endogenous protein flow will therefore have a direct impact on digestible energy commensurate with the composition of the endogenous protein.

Table. 1 Amino acid composition (g/100g amino acids) of various endogenous secretions (Ravindran, 2016).

Amino acid	Pancreatic secretions	Biliary secretions	Mucin
Aspartic acid	12.5	0.4	7.8
Threonine	5.2	0.3	16.4
Serine	6.9	0.3	10.9
Glutamic acid	10.3	1.1	10.1
Proline	5.0	0.3	12.0
Glycine	6.2	95.0	5.5
Alanine	5.4	0.0	7.4
Valine	7.2	0.3	5.9
Isoleucine	5.9	0.2	3.0
Leucine	8.3	0.4	5.7
Tyrosine	5.7	0.2	3.2
Phenylalanine	4.4	0.2	3.5
Histidine	2.6	0.2	1.7
Lysine	5.1	0.3	2.8
Arginine	5.1	0.3	3.5
Methionine	1.1	0.1	0.8
Cystine	1.7	0.6	10.0

Microbial biomass and endogenous protein loss

Microbial protein is an anomaly in endogenous protein measurement and vocabulary. Strictly speaking microbial protein is neither of dietary nor endogenous origin and represents a confounding 'sink' of amino acids that may undergo substantial alteration in composition and characteristics via various bacterial metabolic processes (including conversion of non-protein N to protein-N). Nevertheless,

microbial protein is measured as 'endogenous' via most routine assays and considered as part of both basal and specific losses depending on the experimental procedure used.

Instructively, Miner-Williams et al. (2009) present data that allow conceptual separation of microbial protein from other endogenous protein components. These authors determined that around 61% of the protein in ileal digesta originated from bacterial biomass (the remainder being mucin, sloughed animal cells and digestive enzymes). The amino acid composition of this bacterial biomass is presented in Table 2. Notably, there are some substantial differences between the amino acid composition of e.g. mucin or bile and that of bacterial protein. For example, mucin and bile are dominated by Thr, Ser, Gly, Pro and Cys whereas bacterial protein is dominated by Glu, Asp and Leu. Therefore, changes in the extent of the secretion/abundance and recovery of different sources of endogenous protein will have different implications for the nutritional status of the animal (amino acid digestibility and requirement).

Table 2 Amino acid composition of bacterial biomass (adapted from Miner-Williams et al., 2009).

Amino acid	g/100g of amino acid
Aspartic acid	7.0
Threonine	4.0
Serine	3.6
Glutamic acid	10.9
Proline	3.5
Glycine	3.6
Alanine	4.0
Valine	4.0
Isoleucine	3.0
Leucine	4.4
Tyrosine	2.1
Phenylalanine	2.9
Histidine	1.7
Lysine	3.3
Arginine	3.1
Methionine	1.1
Cystine	1.4

Peptidoglycans can be Relevant Biomass

Noted earlier (Ward and Cowieson, 2017), cell wall fragments constitute a major source of biomass in the intestinal tract – 'intestinal rubbish', if you will. As much as 90% of Gram positive cell walls are comprised of peptidoglycan (PGN), and nearly 75% of the intestinal bacterial can be Gram positive (see Ward and Cowieson, 2017). Finally, 60% of fecal mass is bacterial in nature (O'Hara and Fergus Shanahan, 2006), with nearly 35% being dead or nonviable at the terminal intestine (Apajalahti *et al*., 2003).

The protein from PGN in bacterial cell walls contains a mixture of isomeric D and L forms of amino acids (Friedman, 1999). The amino acid composition of PGN varies considerably with bacterial specie, but regardless of source, the D isomers are of a capricious nutritional value for poultry (Sugahara et al., 1967). Hence, this segment of biomass is disreputable at best. Instead, its significance probably lies in

its sheer quantity on any given day. The abundance of cell wall fragments could have pivotal consequences on animal performance simply by interfering with normal and optimal digestive processes.

Implications and conclusions

Endogenous amino acid losses represent a significant inefficiency in commercial poultry production and should be limited wherever possible. Several strategies to reduce endogenous protein flow have been suggested (Cowieson et al., 2009) and include use of various feed additives such as exogenous enzymes and also feed processing to reduce the presence of antinutrients such as trypsin inhibitors. These strategies are effective in increasing amino acid digestibility (in a pattern that reflects the amino acid composition of the contributory endogenous proteins) and improving net energy.

Potential opportunities to further enhance the efficiency of N cycling in the bird through intentional modification and recovery of the microbial protein pool are exciting and this would be expected to substantially reduce endogenous protein flow in the future (with particular influence on Glu, Asp and Leu). In the case of Glu this may enhance enterocyte metabolism and have a range of adjacent beneficial effects on gut energy partitioning, mucosal metabolism and nutrient absorption (Wu et al., 2014). Thus, in the future, further improvements in the efficiency of N cycling in poultry production may be realized, both through improved retention of dietary amino acids, reduced secretion and loss of endogenous protein and in intentional intervention to recover protein from microbial biomass.

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