

The challenge to reduce crude protein contents of wheat-based broiler diets

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ABSTRACT

The challenge to reduce crude protein (CP) contents of wheat-based broiler diets is both justified and formidable because the performance of broiler chickens offered reduced-CP, wheat-based diets is usually compromised. Moreover, broiler chickens offered wheat-based diets do not accommodate CP reductions as well as do those offered maize-based diets; this appears to stem from the higher protein concentrations and more rapid starch digestion rates of wheat. The higher protein concentrations of wheat than maize result in elevated inclusion levels of non-bound (synthetic, crystalline) amino acids (NBAA). This may be an impediment, because non-bound and protein-bound amino acids are not bioequivalent and intestinal uptakes of NBAA are more rapid than their protein-bound counterparts. This leads to post-enteral amino acid imbalances and the deamination of surplus amino acids, which generates ammonia (NH₃). Because NH₃ is inherently detrimental, it must be detoxified and eliminated as uric acid, which attracts metabolic costs. Moreover, inadequate NH₃ detoxification may seriously compromise broiler growth performance. Also, consideration is given to some intrinsic wheat factors, including soluble non-starch polysaccharides, amylase–trypsin inhibitors and gluten, that may hold relevance. Several strategies are proposed that may enhance the performance of birds offered reduced-CP, wheat-based diets, including capping dietary starch:protein ratios, blending wheat with sorghum, whole-grain feeding in association with phytase, dietary inclusions of L-carnitine and the use of protected or slow-release amino acids. In future research, it should prove instructive to compare different wheats with a wide range of protein contents that, importantly, have been fully characterised for relevant parameters, to ascertain the most appropriate properties. The successful development and adoption of reduced-CP, wheat-based diets would be an enormous advantage for the Australian chicken-meat industry as it would diminish the huge dependence on imported, expensive soybean meal.

Keywords: amino acids, broiler chickens, crude protein, glucose, maize, sorghum, starch, wheat.

Introduction

The reduction of crude protein (CP) concentrations in broiler diets for commercial application in Europe was considered in Garland (2018) and Lemme *et al.* (2022) and some of the attendant advantages have been clearly demonstrated by Brink *et al.* (2022) in birds offered wheat-based diets. The objective of successfully developing reduced-CP diets for Australian chicken-meat production is amply justified. The advantages of reduced-CP broiler diets extend to less environmental pollution via attenuated nitrogen (N) and ammonia (NH₃) emissions, to bird welfare via improved litter quality with lower incidences of foot-pad lesions and related conditions, and to flock health via diminished flows of undigested protein into the hind-gut that fuel proliferation of potential pathogens (Greenhalgh *et al.* 2020a; Alfonso-Avila *et al.* 2022). A reduced-CP diet is one in which dietary protein is reduced by 20 g/kg or more, usually by partially replacing soybean meal with non-bound (synthetic, crystalline) amino acids to meet requirements as non-bound amino acids (NBAA) are effectively an alternative ‘protein’ source to soybean meal (Selle *et al.* 2020). This substitution increases the feed grain

component and, axiomatically, starch concentrations in the diet, which affects starch:protein digestive dynamics in birds offered reduced-CP diets (Liu and Selle 2017). The partial substitution of soybean meal with non-bound amino acids and/or alternative protein feedstuffs is an enormous potential advantage for reduced-CP diets, given the huge dependence of the Australian chicken-meat industry on imported, expensive soybean meal. The objective of this review is to identify the challenges to reducing CP in wheat-based diets and to suggest strategies that may be able to overcome them.

Background

The outcomes of seven evaluations of reduced-CP, wheat-based diets, involving 11 observations, completed in New South Wales, have not been entirely promising. Dietary CP concentrations were reduced by an average of 32 g/kg (174 vs 206 g/kg) in these evaluations, as shown in Table 1. This approach reduced average soybean meal inclusions from 230 to 98 g/kg, or 57.4%, accompanied by increases in NBAA from 7.13 to 24.34 g/kg and average wheat inclusions increased from 553 to 686 g/kg. However, the 32 g/kg reduction in dietary CP compromised growth performance as average weight gains were depressed from 1916 to 1638 g/bird and feed conversion ratio (FCR) was compromised from 1.524 to 1.694 from 13.4 to 35 days post-hatch, as shown in Table 2. The magnitude of the range of these responses is remarkable as evidenced by the tabulated coefficients of variation and outcomes varied from promising in Yin *et al.* (2020) to profoundly compromised weight gain and FCR in Greenhalgh *et al.* (2020b)

and Chrystal *et al.* (2021). Clearly, the factors contributing to these profound variations when CP concentrations of wheat-based diets are reduced demand identification, if at all possible.

Maize is the principal feed grain in global chicken-meat production, but wheat is dominant in Australia with sorghum second. However, wheat-based diets are inferior to maize in two direct comparisons (Chrystal *et al.* 2021; Greenhalgh *et al.* 2022a) as shown in Table 3. In both studies, wheat-based diets supported similar or slightly better growth performance with standard-CP diets, but these advantages were eroded in reduced-CP diets. This pattern was more pronounced in Chrystal *et al.* (2021) than in Greenhalgh *et al.* (2022a), as were the reductions in dietary CP and, paradoxically, increases in relative fat-pad weights were more evident in birds offered maize-based diets. Collectively, these outcomes epitomise the challenge to reduce CP contents of wheat-based broiler diets.

Starch and protein in feed grains

Attempts have been made to identify the shortfalls of wheat in this context (Selle *et al.* 2022a) because of the inconsistent responses in broilers to reduced-CP diets, but it is a complex issue. Feed grain properties and their starch characteristics assume more importance in reduced-CP diets because of their higher inclusion rates. In addition, protein contents and amino acid profiles in a given feed grain affect the extent and pattern of NBAA inclusions in reduced-CP diets.

The relevance of starch:protein disappearance rate ratios from the small intestine to broiler growth performance was

Table 1. Summation of New South Wales reduced-crude protein feeding studies; feeding interval and dietary concentrations of crude protein, wheat, soybean meal and non-bound amino acids (NBAA).

Reference	Days post-hatch	Crude protein (g/kg)		Wheat (g/kg)		Soybean meal (g/kg)		NBAA (g/kg)	
		High	Low	High	Low	High	Low	High	Low
Hilliar <i>et al.</i> (2019)	21–35	209	177	461	561	249	168	4.47	12.88
Hilliar <i>et al.</i> (2020)	21–35	180	165	552	625	177	103	11.43	22.53
	21–35	180	150	552	702	177	24	11.43	35.63
Yin <i>et al.</i> (2020)	14–35	215	190	551	649	247	147	3.94	15.46
	14–35	215	165	551	747	247	47	3.94	26.97
Greenhalgh <i>et al.</i> (2020b)	7–35	197.5	180	607	680	236	174	8.67	17.01
	7–35	197.5	162.5	607	738	236	113	8.67	27.18
Chrystal <i>et al.</i> (2021)	7–35	222	193	525	637	300	177	7.50	26.36
	7–35	222	165	525	751	300	48	7.50	49.39
Dao <i>et al.</i> (2022)	7–35	208.6	183.6	567	696	143	48	3.55	8.51
Greenhalgh <i>et al.</i> (2022a)	21–35	220	180	587	762	223	34	7.30	36.82
Mean	13.4–35.0	206	174	553	686	230	98	7.13	25.34

Table 2. Summation of New South Wales reduced-crude protein feeding studies; performance responses in weight gain and FCR to dietary crude protein concentrations.

Reference	Weight gain (g/bird)			FCR (g/g)		
	High CP	Low CP	Response (%)	High CP	Low CP	Response (%)
Hilliar <i>et al.</i> (2019)	1595	1515	-5.02	1.444	1.535	6.30
Hilliar <i>et al.</i> (2020)	1319	1198	-9.17	1.629	1.661	1.96
	1319	1195	-9.40	1.629	1.614	-0.92
Yin <i>et al.</i> (2020)	2096	2058	-1.81	1.476	1.497	1.42
	2096	2106	0.48	1.476	1.546	4.74
Greenhalgh <i>et al.</i> (2020b)	1958	1451	-25.98	1.684	1.878	11.52
	1958	1010	-48.42	1.684	2.426	44.06
Chrystal <i>et al.</i> (2021)	2403	2386	-0.71	1.453	1.471	1.24
	2403	1549	-35.54	1.453	1.840	26.63
Dao <i>et al.</i> (2022)	1213	1045	-13.85	1.517	1.743	14.90
Greenhalgh <i>et al.</i> (2022a)	2720	2510	-6.89	1.563	1.529	9.06
Mean	1916	1638	-14.29	1.524	1.694	10.99
Median			-9.17			6.30
Standard deviation			±15.782			±13.5227
Coefficient of variation (%)			110.4			123.0

Table 3. Direct comparisons of maize vs wheat as the basis of reduced-crude protein diets in respect of weight gain, FCR and relative abdominal fat-pad weights.

Crude protein (g/kg)	Weight gain (g/bird)			FCR (g/g)			Abdominal fat-pad (g/kg)		
	Maize	Wheat	Difference	Maize	Wheat	Difference	Maize	Wheat	Difference
222 ^A	2214	2403	8.54%	1.453	1.453	0.00%	6.4	6.4	0.00%
193 ^A	2396	2386	-0.42%	1.415	1.471	3.96%	11.1	8.5	-23.4%
165 ^A	2370	1549	-34.6%	1.473	1.840	24.9%	12.8	7.5	-41.4%
220 ^B	2690	2720	1.12%	1.338	1.314	-1.79%	8.02	6.19	-22.8%
180 ^B	2598	2510	-3.39%	1.385	1.433	3.47%	10.16	7.78	-23.4%
Mean	2454	2314	-5.70%	1.413	1.502	6.30%	9.70	7.27	-25.1%

^AChrystal *et al.* (2021).^BGreenhalgh *et al.* (2022a).

demonstrated in Sydenham *et al.* (2017). In this study, maximum weight gain was supported by a starch:protein disappearance rate ratio of 3.59 and minimum FCR by a ratio of 3.88, as predicted by the quadratic relationships detected for weight gain ($r = 0.849$; $P < 0.001$) and FCR ($r = 0.838$; $P < 0.001$). The fundamental concept is that there should be an appropriate balance of amino acids and energy, principally provided as glucose, at sites of protein synthesis to drive efficient skeletal muscle deposition and growth (Liu and Selle 2015).

In reduced-CP diets, dietary starch:protein ratios are axiomatically expanded and this expansion translates to increased starch:protein disappearance-rate ratios in birds. For example, Chrystal *et al.* (2020) evaluated maize-based diets with CP contents of 200, 188, 172 and 156 g/kg in

which analysed dietary starch:protein ratios expanded from 1.48 to 1.76, 2.05 and 2.54 respectively. In birds, distal jejunal starch:protein disappearance-rate ratios linearly increased from 2.08 to 3.17, with a similar increase from 2.01 to 3.06 in the distal ileum. However, distal jejunal ratios were negatively correlated to weight gain ($r = -0.422$; $P = 0.025$) and positively correlated to FCR ($r = 0.835$; $P < 0.001$), which deteriorated from 1.495 to 1.500, 1.522 and 1.629 with dietary CP reductions for an overall 8.96% decline in efficiency of feed conversion. Also, distal ileal ratios were positively correlated with FCR ($r = 0.767$; $P < 0.001$). Relative abdominal fat-pad weights increased by 62.7% (12.40 vs 7.62 g/kg) as dietary CP was reduced from 200 to 156 g/kg and FCR increased quadratically ($r = 0.752$; $P < 0.001$) in relation to increased fat deposition.

This, and similar outcomes, led to the approach of ‘capping’ dietary starch:protein ratios, as discussed later in this review.

Starch and glucose

The digestion-rate constant of wheat starch (0.035) is more rapid than those of maize (0.017) and sorghum (0.018) under *in vitro* conditions (Giuberti *et al.* 2012) and similar patterns have been reported in broiler chickens (Selle *et al.* 2021a), where the value for wheat starch (0.117) was again more rapid than maize (0.086) and sorghum (0.075). The provision of some slowly digestible starch in broiler diets has been shown to be advantageous in broilers offered atypical diets containing either (rapidly digestible) wheat starch or (slowly digestible) pea starch by Herwig *et al.* (2019). A similar evaluation with reduced-CP diets could prove instructive. It is also possible that slowly digestible starch spares amino acids from catabolism in the gut mucosa for the provision of energy to drive digestive processes (Enting *et al.* 2005). The mean proportion of wheat starch digested in the proximal jejunum (by definition rapidly digestible starch), as opposed to the entire small intestine, was 38.2% in broilers offered diets based on six classes of Canadian wheats in Karunaratne *et al.* (2018). However, the proportion of rapidly digestible starch ranged from 25.0% to 51.4% and this variation may be contributing to the inconsistent

responses of broiler chickens following CP reductions in wheat-based diets.

Protein and amino acids

The protein contents and amino acid profiles of wheat and maize are different. In one Australian survey (Bryden *et al.* 2009), the mean protein concentration of 27 wheat samples was 115.5 g/kg \pm 22.32, as opposed to 80.0 g/kg \pm 3.56 in seven maize samples. Thus, the protein contents of wheat are both higher and more variable than maize. The amino acid profiles of the two feed grains from the same survey are shown in Table 4. There are considerably higher concentrations of glutamic acid in wheat than maize, but the reverse applies to leucine, methionine, threonine, alanine and aspartic acid. Importantly, protein contents and amino acid concentrations in a given feed grain will influence the extents of NBAA inclusions and the balance of protein-bound to non-bound amino acids in reduced-CP diets. The higher protein content of wheat dictates that there will be higher NBAA inclusions in wheat-based, reduced-CP diets than in corresponding maize-based diets. This is illustrated in Table 5, where 165 g/kg CP, maize-based diets contained 38.49 g/kg NBAA, but the corresponding wheat-based diets contained 49.39 g/kg NBAA. However, this is probably disadvantageous, simply because it is unlikely that non-bound and protein-bound amino acids are bioequivalent, which

Table 4. Protein and amino acid concentrations in wheat and maize expressed in absolute and proportional terms and relative to lysine (100) [adapted from Bryden *et al.* (2009)].

Item	Wheat (n = 27)			Maize (n = 7)		
	Concentration (g/kg as-is)	Proportion (%)	Relative to lysine (100)	Concentration (g/kg as-is)	Proportion (%)	Relative to lysine (100)
Protein	115.5			80.0		
Arginine	5.65	5.4	158	3.97	5.2	157
Histidine	3.10	2.9	86	2.43	3.2	96
Isoleucine	4.58	4.3	128	3.21	4.2	127
Leucine	8.44	8.0	235	10.80	14.3	427
Lysine	3.58	3.4	100	2.53	3.3	100
Methionine	1.57	1.5	44	1.46	1.9	58
Phenylalanine	5.81	5.5	162	4.27	5.6	169
Threonine	3.71	3.5	103	3.43	4.5	136
Tryptophan	1.33	1.3	37	0.58	0.8	23
Valine	5.43	5.1	151	4.33	5.7	171
Alanine	4.46	4.2	124	6.61	8.7	261
Aspartic acid	6.20	5.9	173	5.70	7.5	225
Glutamic acid	36.74	34.8	1025	16.26	21.5	643
Glycine	5.10	4.8	142	3.30	4.4	130
Serine	6.51	6.2	182	4.16	5.5	165
Tyrosine	3.23	3.1	90	2.69	3.6	106
Total	105.44	100.0		75.73	100.0	

Table 5. The impact of CP reductions (dietary CP 222, 193, 165 g/kg) on the composition of maize- and wheat-based diets [adapted from [Chrystal et al. \(2021\)](#)].

Item	Maize (81 g/kg CP)			Wheat (107 g/kg CP)		
	222 g/kg	193 g/kg	165 g/kg	222 g/kg	193 g/kg	165 g/kg
Feed grain	511	615	721	525	637	751
Soybean meal	334	228	113	300	177	48
Canola seed	60	60	60	60	60	60
Non-bound amino acids	7.23	19.47	38.49	7.50	26.36	49.39

probably results in post-enteral amino acid imbalances ([Selle et al. 2022b](#)).

Protein and starch digestion and intestinal uptakes of amino acids and glucose

Intestinal uptake rates of nutrients, including amino acids and glucose, are pivotal to the growth performance of broiler chickens ([Croom et al. 1999](#)). Reduced-CP diets contain higher starch concentrations than do standard broiler diets; however, there is evidence to suggest that glucose may impede intestinal uptakes of amino acids ([Murer et al. 1975](#); [Alvarado and Robinson 1979](#); [Stevens et al. 1984](#); [Vinardell 1990](#)). To some extent, these findings may reflect the more general impact of starch–protein interactions, which are complex ([Chao et al. 2018](#)).

However, dietary CP of maize-based broiler diets was reduced from 213 to 183 g/kg mainly by increasing maize starch (0–464 g/kg) at the expense of maize grain (465–114 g/kg) in [Moss et al. \(2018\)](#). NBAA inclusions were increased from 3.54 to 17.82 g/kg and soybean meal inclusions were slightly reduced from 370 to 340 g/kg. Interestingly, apparent starch digestibility coefficients were negatively correlated with digestibility coefficients of four amino acids in proximal jejunum, nine in distal jejunum, 12 in proximal ileum and 11 amino acids in distal ileum to significant extents. In the proximal ileum, 8 of the 12 relevant amino acids (arginine, isoleucine, lysine, methionine, threonine, valine, glycine, serine) were included in the 183 g/kg CP diet as both non-bound and protein-bound entities. However, the balance of four amino acids (histidine, leucine, phenylalanine, glutamic acid) was present only as protein-bound entities. Thus, it appears that glucose was impeding intestinal uptakes of both protein-bound and non-bound amino acids in [Moss et al. \(2018\)](#). Non-bound or monomeric amino acids are absorbed via an array of some 23 Na⁺-dependent and Na⁺-independent transporters with overlapping amino acid affinities ([Hyde et al. 2003](#)). Some monomeric amino acids and glucose are co-absorbed via the Na⁺-dependent transporter, SGLT-1 ([Wright 1993](#); [Shibata et al. 2019](#)). Alternatively, di- and tri-peptides derived from intact protein digestion are mainly absorbed via the peptide

transporter, PepT-1 ([Zwarycz and Wong 2013](#); [Wang et al. 2021](#)). Importantly, intestinal uptakes of amino acids as di- and tri-peptides, or oligopeptides, are dominant and energetically more efficient than are intestinal uptakes of monomeric amino acids ([Daniel 2004](#); [Gilbert et al. 2008](#)). Inclusions of NBAA were increased from 3.54 to 17.82 g/kg with the dietary CP reduction from 213 to 183 g/kg in the [Moss et al. \(2018\)](#) study. Also, the mean proportion of dietary NBAA inclusions of total analysed amino acid concentrations ranged from 3.65% (arginine) to 72.3% (methionine) across nine amino acids. This raises the possibility that NBAA were competing for co-absorption with Na via SGLT-1 to the detriment of amino acid digestibilities. The other possibility is that starch may have impeded the digestive conversion of intact protein to oligopeptides and their intestinal uptakes via PepT-1, again to disadvantage amino acid digestibilities in the [Moss et al. \(2018\)](#) study.

Apparent jejunal and ileal amino acid digestibility coefficients in broilers offered maize- or wheat-based diets were directly compared by [Greenhalgh et al. \(2022a\)](#). Wheat supported inferior average digestibility coefficients of 16 amino acids in distal jejunum by 5.24% (0.741 vs 0.782) in 220 g/kg CP diets but this increased to a discrepancy of 9.94% (0.734 vs 0.811) in 180 g/kg CP diets. A similar pattern was observed in the distal ileum where the shortfall of 2.38% (0.862 vs 0.883) in 220 g/kg CP diets expanded to 6.67% (0.840 vs 0.900) in 180 g/kg CP diets. Soluble non-starch polysaccharides (NSP) are one possible cause of the relative inferiority of wheat. Wheat contains higher concentrations of soluble NSP than does maize by 25 vs 9 g/kg on a dry-matter basis ([Knudsen 1997](#)). While all diets contained NSP- and phytate-degrading feed enzymes in the [Greenhalgh et al. \(2022a\)](#) study, [Munyaka et al. \(2016\)](#) reported that wheat generated higher average gut viscosities in broiler chickens than did maize by 61.3% (3.08 vs 1.91 MPa), irrespective of inclusions of dietary NSP-degrading enzymes. Also, [Choct et al. \(1996\)](#) found that NSP-enriched diets promoted small intestinal fermentation and considered this to contribute to the anti-nutritive effects of soluble NSP. Xylanase inclusions in either wheat- or maize-based broiler diets had differing impacts on the composition of caecal microbiota in [Wang et al. \(2021\)](#). Thus, it is possible that (1) higher gut viscosities in birds

offered wheat-based diets impeded protein digestion and amino acid absorption and (2) increased microbial fermentation along the small intestine depressed apparent amino acid digestibilities by increasing concentrations of small intestinal microbial amino acids in Greenhalgh *et al.* (2022a).

Support for this is provided by the relative proportions of dietary, endogenous and microbial amino acids in distal ileal digesta estimated by the Duvaux *et al.* (1990) model in the Greenhalgh *et al.* (2022a) study. The transition from 220 to 180 g/kg CP diets modestly increased the proportion of microbial amino acids from 22.1% to 24.4% in birds offered maize-based diets. However, the same transition increased the proportion of microbial amino acids in wheat-based diets from 29.2% to 38.6%. Moreover, estimated microbial amino acid proportions in distal ileal digesta were negatively correlated with apparent digestibility coefficients of all 16 amino acids assessed, to highly significant extents across the entire study.

A second possible factor is amylase–trypsin inhibitors (ATI), which are abundant in the soluble albumin fraction of wheat (Geisslitz *et al.* (2022)). Potentially, wheat ATI could increase pancreatic secretions of amylase and trypsin to counteract the inhibition, which would increase endogenous flows of relevant amino acids and could even compromise starch and protein digestibilities. However, whether wheat ATI are potent anti-nutritive factors in broiler chickens is problematic. Macri *et al.* (1977) reported that albumin amylase inhibitors from wheat depressed broiler growth performance, but only when administered as cellulose-coated microgranules resistant to pepsin activity. Unprotected wheat albumins did not compromise growth performance, which implies that wheat ATI may be vulnerable to pepsin activity and/or low pH in the gizzard. Nevertheless, further investigations into wheat ATI in broilers are probably justified, given their potential to affect starch and protein digestion.

A third possible factor is the gluten content of wheat, which is an insoluble storage protein comprising ~80–85% of the wheat proteome and is subdivided into gliadins and glutenins (Van der Borgh *et al.* 2005). Gliadin has the capacity to compromise the integrity of small intestinal tight junctions by inducing inflammation and increasing intestinal permeability (De Punder and Pruijboom 2013). Moreover, dietary additions of gluten have been reported to depress broiler growth performance (Afshar and Moslehi 2006; Fang *et al.* 2017; Kang *et al.* 2019). A typical 120 g/kg protein wheat would contain in the order of 100 g/kg gluten and the increase in wheat inclusion level of 133 g/kg, from 553 to 686 g/kg (Table 1), would increase gluten by about 13.3 g/kg in the reduced-CP diet. Given that Fang *et al.* (2017) found that the addition of 20 g/kg gluten to broiler diets depressed weight gain (8.37%), feed intake (6.21%) and FCR (2.65%), then the increased gluten concentrations in reduced-CP, wheat-based diets could assume importance.

Intestinal uptake rates of protein-bound versus non-bound amino acids

It is improbable that protein-bound and non-bound amino acids are bioequivalent in broiler chickens (Selle *et al.* 2022b) and elevated inclusions of NBAA increase the pertinence of this issue in reduced-CP, wheat-based diets. From first principles (Wu 2009), NBAA are more rapidly absorbed along the small intestine. Synthetic *d,l*-methionine was made available for animals in 1950s; however, Canolty and Nasset (1975) subsequently found that cumulative plasma methionine concentrations in rats offered synthetic methionine at 15, 30, 60 and 120 min post-administration were 2.75 time higher (858 vs 312 $\mu\text{mol/L}$) than in rats receiving methionine only from intact protein. Of direct relevance is that intestinal uptakes of NBAA are more rapid than are their protein-bound counterparts in broiler chickens. This was unequivocally demonstrated by Liu *et al.* (2013), where standard CP, sorghum-based diets contained 3.7 g/kg lysine (as lysine HCl), 3.4 g/kg *d,l*-methionine and 1.3 g/kg threonine or a total of 8.4 g/kg NBAA. It may be deduced from this study that the mean digestion constant of 13 protein-bound amino acids was $2.35 \times 10^{-2} \text{ min}^{-1}$ as opposed to $8.78 \times 10^{-2} \text{ min}^{-1}$ for non-bound lysine and $8.49 \times 10^{-2} \text{ min}^{-1}$ for non-bound methionine. Therefore, intestinal uptakes of NBAA were nearly four times more rapid than were those of protein-bound amino acids. More recently, Zamani *et al.* (2021) reported that 60-min, post-prandial methionine concentrations in plasma from broiler chickens were higher in diets supplemented with either a methionine dipeptide (163 ng/ μL) or *d,l*-methionine (155 ng/ μL) by more than a four-fold, factor than were those in the control, maize-soy diet (36 ng/ μL), which did not contain any non-bound methionine. Thus, bioequivalency is effectively precluded by these differences in intestinal uptake rates between protein-bound and non-bound amino acids and this disparity has post-enteral consequences.

Hepatic deamination of amino acids and ammonia

The negative impacts of NH_3 generated systemically by hepatic deamination of amino acids and by microbial fermentation along the digestive tract merit attention. Importantly, the intravenous LD_{50} of ammonium acetate in broilers (2.72 mmol/kg) was half that in mice (5.64 mmol/kg); thus, NH_3 is noticeably more toxic in poultry than in mice (Wilson *et al.* 1968) and, presumably, other mammalian species. The negative impacts of atmospheric NH_3 in poultry are well established (Han *et al.* 2021), but are essentially irrelevant to this review.

Protein turnover is a dynamic process involving both protein deposition and protein degradation taking place mainly in skeletal muscles and a positive balance represents

protein accretion, or net protein synthesis and growth (Swick 1982; Tesseraud *et al.* 2000). Nevertheless, protein degradation is a continuously ongoing process generating endogenous NH₃; however, post-enteral imbalances between non-bound and protein-bound amino acids and the deamination of surplus NBAA are likely to trigger additional NH₃ concentrations in broiler chickens offered reduced-CP diets (Selle *et al.* 2022b). Amino acids exceeding requirements for protein synthesis are rapidly catabolised (Brosnan 2003); therefore, the likelihood is that NBAA will undergo catabolism, or post-prandial oxidation (Schreurs *et al.* 1997) to greater extents than do protein-bound amino acids because NBAA are more rapidly absorbed. The post-prandial catabolism of non-bound leucine was shown to be greater than that of leucine bound in albumen in rats by Nolles *et al.* (2009). The post-prandial oxidation of egg white protein versus a corresponding blend of NBAA was determined via ¹³CO₂ breath tests in this study. Post-prandial oxidative losses of non-bound leucine were significantly higher than those of protein-bound leucine by an approximate factor of 1.52 (24.8 vs 16.3%) after a short adaptation period. In a similar earlier study, Bujko *et al.* (2007) found that cumulative oxidative losses of non-bound leucine (26.9%) exceeded those of protein-bound leucine (16.0%) over a post-prandial period of 300 min in rats. Thus, NBAA are more likely to be lost to post-prandial oxidation from post-enteral amino acid imbalances triggered by their more rapid intestinal uptakes than are protein-bound amino acids.

In an unpublished study completed on the Camden Campus, CP concentrations of broiler diets were reduced from 210 to 190 and 170 g/kg, with corresponding increases in NBAA from 15.1 to 39.0 and 55.4 g/kg. Reducing dietary CP linearly increased ($r = 0.528$; $P = 0.024$) plasma NH₃ concentrations by 17.1%, from 1.87 to 2.19 µg/g. This outcome accords with the proposal that NBAA are more likely to generate NH₃ following their post-enteral deamination.

In addition, NH₃ is generated along the digestive tract by microbial fermentation and is readily absorbed in poultry (Yutaka and Chitose 1986). Concentrations of NH₃ in the caeca (6–10 mM) of poultry exceeded those in the small intestine (1.2–1.9 mM) in poultry in Prior *et al.* (1974) and Isshiki (1980) found that caecal concentrations of NH₃-N (11.58 mg/kg) were substantially higher than those in the distal ileum (1.93 mg/kg) of broiler chickens. These differences are largely caused by urinary uric acid entering the coprodeum of the large intestine and entering the caeca via reverse peristalsis, to be degraded to NH₃ by microbial fermentation (Gilbert *et al.* 2018). Interestingly, Visek (1978) proposed that the suppression of NH₃ concentrations derived from microbial fermentation may be the principal mode of action of the so-called antibiotic growth promotants.

In an instructive study, Okumura and Tasaki (1969) determined the effect of dietary protein concentration on NH₃ and uric acid plasma concentrations in chickens for up to 360 min post-prandially. Dietary protein concentrations

were increased by casein inclusions ranging from 0 to 400 g/kg in a stepwise manner. Increasing dietary protein increased average plasma NH₃ concentrations from 0.34 up to 0.49 NH₃-N mg/100 mL and plasma uric acid concentrations from 5.6 up to 15.0 mg/100 mL. However, it may be deduced from the Okumura and Tasaki (1969) study that NH₃ to uric acid plasma concentration ratios increased quadratically ($r = 0.899$; $P = 0.037$) as protein contents were reduced. In a subsequent study, Karasawa (1986) infused ¹⁵N-ammonia into chickens offered either 50 or 200 g/kg CP diets. The infused NH₃ was almost entirely excreted as uric acid in birds offered 200 g/kg CP diets. In contrast, infused NH₃ was excreted as both NH₃ and uric acid in birds offered 50 g/kg CP diets. These outcomes suggest that low dietary CP concentrations limit the capacity of birds to detoxify NH₃, which is consistent with the proposal that one molecule of glycine and aspartic acid and two molecules of glutamine are required for the conversion of four NH₃ molecules into uric acid, as advanced by Mapes and Krebs (1978).

Ammonia detoxification

As NH₃ is inherently noxious, detoxification is demanded (Stern and Mozdziak 2019). Briefly, this is achieved first by the condensation of NH₃ with glutamic acid to generate glutamine in a reaction catalysed by glutamine synthetase (Hakvoort *et al.* 2017). Second, glutamine enters the Krebs uric acid cycle where it is converted to uric acid, which requires an input of one mole of glycine for every mole of uric acid excreted (Salway 2018). An average of 49.2% of dietary glycine, ranging from 25.0% to 80.9%, was partitioned into the Krebs uric acid cycle in the Chrystal *et al.* (2021) study. This was from a retrospective estimate that does not make an allowance for any biosynthesis of glycine (Selle *et al.* 2021b). In addition, NH₃ detoxification attracts metabolic costs in terms of energy inputs. The synthesis and excretion of uric acid to void NH₃-N in urine generates a minimal energy cost of 64.7 kJ/g N excreted as uric acid in poultry (van Milgen 2021). Uric acid concentrations in broiler excreta were determined in Selle *et al.* (2021b) during the total collection period, to determine nutrient utilisation. It may be deduced from these data that the proportion of dietary energy intake partitioned to uric acid synthesis and excretion amounted to 2.26% of gross energy (17.21 MJ/kg GE) or 2.98% of metabolisable energy (13.06 MJ/kg AME).

Detoxification of NH₃ is usually adequate as it is converted into and excreted as uric acid; however, increasing plasma NH₃ concentrations have been associated with compromised performance in three broiler studies (Namroud *et al.* 2008; Ospina-Rojas *et al.* 2013, 2014), which suggests that NH₃ was not being adequately detoxified and excessive systemic NH₃ concentrations were depressing growth performance. In addition, excreta NH₃ concentrations from birds offered

maize–soy diets containing 163, 147 and 132 g/kg CP with four levels of glycine equivalents from 7 to 21 days post-hatch were determined by Hofmann *et al.* (2019). Dietary NBAA inclusions ranged from 17.5 to 67.3 g/kg across 12 dietary treatments. Excreta NH₃ concentrations were indicative as they were negatively correlated with weight gain ($r = -0.761$; $P = 0.004$), feed intake ($r = -0.754$; $P = 0.005$) and gain to feed ($r = -0.753$; $P = 0.005$). Moreover, a quadratic relationship ($r = 0.978$; $P < 0.0001$) may be derived between dietary NBAA inclusions and excreta NH₃ concentrations, as shown in Fig. 1. The regression equation predicts that when NBAAA inclusions exceed 31.1 g/kg, excreta NH₃ concentrations increase in a quadratic manner. Reductions in dietary CP were investigated by Brink *et al.* (2022), in which finisher broiler diets were formulated to 195, 180 and 165.6 g/kg CP, which contained 5.4, 10.1 and 16.9 g/kg NBAA respectively. The transition from 195 to 165.6 g/kg CP significantly reduced excreta N concentrations by 20.3% (255 vs 320 g/kg) and uric acid by 36.7% (50.1 vs 79.2 g/kg) at 39 days post-hatch. However, total NH₃-N in excreta increased numerically by 9.05% (2.29 vs 2.10) in absolute terms or by a factor of 1.36 from 0.66% to 0.90% as a proportion of total excreta N. Collectively, these outcomes indicate that NBAA inclusions may become excessive and contribute to inadequate detoxification, and the resultant NH₃ overload compromises growth performance.

Investigations into NH₃ overload should include simultaneous determinations of NH₃ and uric acid concentrations in systemic plasma, digesta from segments of the small and large intestine, excreta and hepatic tissue in broiler chickens offered diets with different CP contents. Both NH₃ and uric acid liver concentrations were determined in

Okumura and Tasaki (1969). Also, allied determinations of orotic acid in excreta may be instructive because urinary excretion of orotic acid becomes elevated when NH₃ concentrations exceed hepatic detoxification capacity of the ureotelic species (Vissek 1984). Thus, orotic acid may be a valuable biomarker in NH₃ overload investigations in broiler chickens if it is similarly indicative in avian species.

Future directions

It should prove instructive to compare a range of fully characterised wheats to ascertain the most appropriate properties in the context of reduced-CP diets. Valuable data could include determinations of *in vitro* starch digestion rates and starch pasting profiles by rapid visco-analysis (RVA). There would appear to be a ceiling on the extent to which starch concentrations and NBAA inclusions may be increased in reduced-CP, wheat-based diets before growth performance is compromised. Thus, future research should identify alternative approaches to reduce CP in wheat-based diets.

Capping dietary starch:protein ratios

Capping, or condensing, dietary starch:protein ratios in reduced-CP diets has shown some advantages in both maize-based (Greenhalgh *et al.* 2022b) and wheat-based (Greenhalgh *et al.* 2020b) diets. Capping dietary starch:protein ratios in 197.5 g/kg CP, wheat-based diets significantly increased weight gain by 10.4% (2161 vs 1958 g/bird), numerically improved FCR by 4.04% (1.616 vs 1.684) from 7 to 35 days post-hatch and fractionally reduced fat-pad weights by 2.70% (8.15 vs 8.37 g/kg). In both studies, capping dietary starch:protein ratios was achieved by substituting soybean meal (505 g/kg CP) with ‘full-fat’ soy (362 g/kg CP) procured from the same provider. Given the Australian situation, substituting soybean meal with canola meal rather than ‘full-fat’ soy would be a preferable approach and certainly merits evaluation.

Blended feed grain diets

Reduced-CP diets based on an equal blend of wheat and maize were evaluated in straight-run Cobb 500 birds kept in floor-pens from 1 to 41 days post-hatch by Maynard *et al.* (2021). The dietary treatments were formulated to contain 215, 190 and 165 g/kg CP. No significant differences were observed in weight gain (3186 vs 3145 g/bird) or feed intake (4873 vs 5009 g/bird) between the high- and low-CP diets, but FCR was significantly compromised by 3.43% (1.597 vs 1.544) following the transition from 215 to 165 g/kg CP diets. Nevertheless, soybean meal consumption was reduced by 62.7% (500 vs 1339 g/bird) by the same transition. Also, the transition from 215 to 190 CP diets did not statistically influence growth performance but did reduce

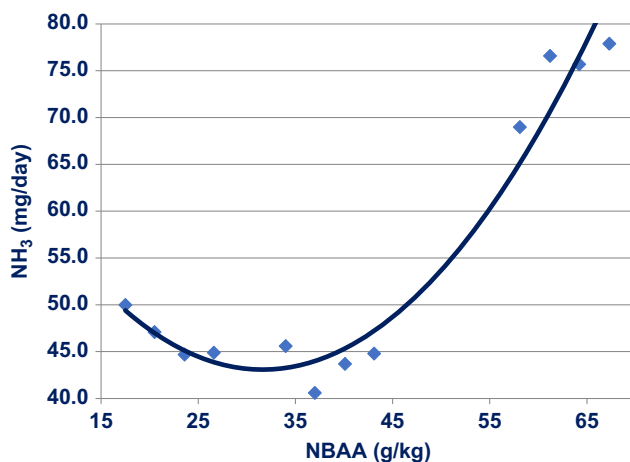


Fig. 1. Quadratic relationship ($r = 0.978$; $P < 0.0001$) between dietary non-bound amino acid (NBAA) inclusions (g/kg) and excreta ammonia (NH₃) concentrations (mg/day) from 7 to 21 days post-hatch, where $y = 74.63 + 0.032 \times \text{NBAA}^2 - 1.993 \times \text{NBAA}$. Adapted from Hofmann *et al.* (2019).

soybean meal consumption by 30.9% (925 vs 1339 g/bird). Compared with 'wheat only', an equal wheat and maize blend is probably advantaged by a lower protein content, which leads to decreased NBAA inclusions, coupled with slower starch digestion rates. Maize is not a practical option in Australia, but sorghum is similar in respect of protein content, amino acid profile and starch digestion rate. Moreover, an equal wheat–sorghum blend has shown some promise relative to wheat in a branched-chain amino acid feeding study (Greenhalgh *et al.* 2022a).

Whole-grain feeding

With wheat-based diets, the inclusion of some whole grain, either pre- or post-pelleting, has become the standard practice in Australia. Whole-grain feeding (WGF) has been evaluated in the context of reduced-CP diets by Yin *et al.* (2020), in which WGF had no impact on growth performance but WGF did generate significantly lighter relative abdominal fat-pad weights. In this study, dietary inclusions of 150 and 250 g/kg whole wheat increased relative gizzard weights by 36.5% and 53.8% respectively, which is a hallmark WGF response; however, the capacity of birds to accommodate dietary CP reductions was not enhanced. Nevertheless, further investigations into WGF in association with reduced-CP, wheat-based diets do appear justified on the basis of the lighter relative abdominal fat-pad weights reported by Yin *et al.* (2020). In addition, Moss *et al.* (2019) concluded that the combination of phytase inclusions and 'pre-pellet' cracked maize advantaged the performance of broilers offered reduced-CP diets. The dietary inclusion of some cracked maize (150 and 300 g/kg) prior to steam-pelleting is somewhat analogous to whole grain feeding to stimulate gizzard function and should, in turn, enhance phytase efficacy as the gizzard is the principal site of enzymatic phytate degradation (Truong *et al.* 2016).

L-carnitine

L-carnitine is a quaternary amino compound that is present in relevant feedstuffs and may be biosynthesised from its amino acid precursors, lysine and methionine, which involves methylation of only protein-bound lysine (Rebouche 2004). Of relevance is that L-carnitine promotes fat mobilisation via the so-called 'carnitine shuttle', which transfers long-chain fatty acids across the barrier of inner mitochondria membranes to undergo β -oxidation and fat is partitioned to energy production (Eder 2009). L-carnitine has displayed promise in limiting lipid deposition in some feeding studies (Rabie *et al.* 1997a, 1997b; Rabie and Szilágyi 1998; Xu *et al.* 2003), although responses of broiler chickens to dietary L-carnitine inclusions are not consistent. However, Rabie *et al.* (1997a) included 50 mg/kg L-carnitine in 180 g/kg CP diets, which reduced relative abdominal fat-pad weights by 20.5% (22.9 vs 28.8 g/kg), with an

associated improvement in FCR by 5.48% (2.07 vs 2.19), from 18 to 53 days post-hatch. Also, 50 mg/kg L-carnitine reduced relative abdominal fat-pad weights by 16.2% (9.8 vs 11.7 g/kg) in broiler chickens at 49 days post-hatch in Xu *et al.* (2003). The inconsistency of L-carnitine responses may stem from variations in its concentrations in relevant feedstuffs, but its potential to depress lipid deposition certainly holds relevance in reduced-CP diets.

Moreover, L-carnitine is protective against NH_3 toxicity (Kloiber *et al.* 1988). In Greenhalgh *et al.* (2022c), broiler chickens were offered sorghum-based diets with CP concentrations of 220, 190 and 160 g/kg from 7 to 33 days post-hatch. The transition from 220 to 160 g/kg CP diets severely depressed weight gain (38.2%), feed intake (25.8%) and FCR (20.3%). However, the 75 mg/kg L-carnitine inclusion in 160 g/kg CP diets increased weight gain by 15.0% (1580 vs 1374 g/bird; $P < 0.001$), feed intake by 8.34% (2403 vs 2218 g/kg; $P < 0.001$) and improved FCR by 5.82% (1.521 vs 1.615; $P = 0.003$), but similar L-carnitine responses were not observed in 190 and 220 g/kg CP diets. These outcomes are consistent with the possibility that L-carnitine was counteracting the impacts of NH_3 intoxication in birds offered 160 g/kg CP diets loaded with 51.02 g/kg NBAA.

'Slow-release' NBAA

Finally, the availability of protected or slow-release NBAA with intestinal uptake rates more akin to protein-bound amino acids would almost certainly be advantageous for the acceptance of reduced-CP diets. The obvious caveat is the economic feasibility of slow-release NBAA. However, it has been reported that lipid-encapsulated lysine and methionine were both more effectively utilised in broiler chickens than were non-bound entities (Sun *et al.* 2020).

Conclusions

Clearly, the challenge to reduce CP contents of wheat-based broiler diets is formidable. However, it should prove instructive to compare different wheats with a wide range of protein contents that, importantly, have been fully characterised in respect of amino acid profiles, concentrations of soluble NSP, ATI and gluten. In addition, RVA starch pasting profiles and *in vitro* starch digestion rates may prove valuable indicators. The potential advantages that the successful development and adoption of reduced-CP, wheat-based diets hold for the Australian chicken-meat industry certainly merit well directed and well funded research to achieve this objective.

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