# Rapid protein disappearance rates along the small intestine advantage poultry performance and influence the post-enteral availability of amino acids

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(Submitted 21 June 2017 – Final revision received 9 October 2017 – Accepted 19 October 2017 – First published online 5 December 2017)

# Abstract

NS British Journal of Nutrition

A foundation diet, an intermediate blend and a summit diet were formulated with different levels of soyabean meal, casein and crystalline amino acids to compare 'slow' and 'rapid' protein diets. The diets were offered to male Ross 308 chicks from 7 to 28 d post-hatch and assessed parameters included growth performance, nutrient utilisation, apparent digestibility coefficients and disappearance rates of starch and protein (N) in four small intestinal segments. Digestibility coefficients and disappearance rates of sixteen amino acids in three small intestinal segments and amino acid concentrations in plasma from portal and systemic circulations from the foundation and summit diets were determined. The dietary transition significantly accelerated protein (N) disappearance rates in the distal jejunum and ileum. The transition from foundation to summit diets significantly increased starch digestibility coefficients in the ileum and disappearance rates in all four small intestinal segments. These starch responses were associated with significant enhancements in nutrient utilisation. The dietary transition linearly increased those of four amino acids relative to the foundation diet to significant extents. Plasma concentrations of five amino acids were higher in the portal than systemic circulations. Rapid protein disappearance rates advantaged poultry performance and influenced post-enteral availability of amino acids. If the underlying mechanisms are to be identified, further research into the impact of protein digestive dynamics on broiler performance is required but appears justified.

Key words: Amino acids: Energy: Poultry: Proteins: Starch

Protein digestion rates in human nutrition have received atten $tion^{(1-3)}$ ; however, the likelihood is that the focus on protein digestion rates in the formulation of least-cost diets for rapidly growing broiler chickens has been insufficient. Here the current emphasis is more on static, ileal amino acid digestibility coefficients rather than kinetics of protein and amino acid digestion. Moreover, it has been proposed that the rates of digestion of protein and absorption of amino acids are surpassed by the rates of starch digestion and glucose absorption and that this asynchrony in digestive dynamics compromises the performance of broiler chickens<sup>(4)</sup>. If so, it follows that the provision of slowly digestible starch will enhance broiler performance, and this concept has been validated to some extent<sup>(5)</sup>. However, it also follows that the provision of rapidly digestible protein should enhance broiler performance in a reciprocal manner. Thus, the objective of this study was to examine the hypothesis that the dietary provision of rapidly digestible protein enhances the performance of broiler chickens.

To this end a conventional, maize-soya 'slow-protein' foundation diet and a 'rapid-protein' summit diet were formulated and an equal blend of these diets constituted an intermediate dietary treatment. The rationale was that the partial replacement of soyabean meal with casein and additional crystalline amino acids in the summit diet would accelerate protein digestion rates. Decades ago, it was demonstrated in rats that concentrations of lysine and methionine derived from casein were higher in the portal blood in comparison with those from soya flour<sup>(6,7)</sup>. Both studies indicated that casein is a more rapidly digested and absorbed source of protein than soya and casein generates higher concentrations of amino acids in the portal circulation. Crystalline (or synthetic) amino acids do not undergo digestion and are directly available for absorption in the upper small intestine and appear in the portal circulation more rapidly than protein-bound amino acids<sup>(8)</sup>. Thus, crystalline amino acids are, axiomatically, rapid sources of 'protein'.

The absorption of nutrients is considered to be a more important rate-limiting factor on the growth performance of broiler chickens than their digestion<sup>(9)</sup>. However, following their absorption, amino acids may be subject to catabolism in small intestinal enterocytes for energy provision to the gut<sup>(10)</sup>

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Abbreviations: AIA, acid insoluble ash; AME, apparent metabolisable energy; DI, distal ileum; ME:GE, metabolisable energy:gross energy; PI, proximal ileum.

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https://doi.org/10.1017/S0007114517003257 Published online by Cambridge University Press

and this is a partial determinant of their entry into the portal circulation and their post-enteral availability for protein accretion. Therefore, plasma concentrations of free amino acids in the portal (anterior mesenteric vein) and systemic (brachial vein) circulations were determined in birds offered the foundation and summit diets. It has been demonstrated that concentrations of six essential and three non-essential amino acids in plasma taken from the anterior mesenteric vein were significantly correlated (P < 0.05) with feed conversion ratios (FCR) in broiler chicks from 7 to 27 d post-hatch<sup>(11)</sup>. Therefore, it was considered that amino acid concentrations in the portal and systemic circulations would be instructive in the present study.

#### Methods

# Diet preparation

A 'slow-protein' foundation and a 'rapid-protein' summit diet were formulated to be iso-energetic (12.97 MJ/kg) and to meet recommended nutrient specifications as shown in Table 1.

Table 1. Composition and calculated nutrient specifications of foundation,
intermediate and summit experimental diets

Items (g/kg)	Foundation diet	Intermediate diet	Summit diet
Maize	501.5	573.1	644·7
Soyabean meal	251.8	166-9	82.0
Rapeseed meal	150.0	149.4	148.8
Casein	0.00	25.0	50·0
Sunflower oil	45.64	29.46	13.27
Lys hydrochloride	1.27	1.78	2.28
Met	1.61	1.80	1.98
Thr	0.17	0.63	1.08
Trp	0.00	0.26	0.51
Arg	0.00	2.35	4.70
lle	0.00	0.60	1.20
Sodium chloride	1.99	1.84	1.69
Sodium bicarbonate	2.58	2.89	3.19
Limestone	6.94	7.04	7.14
Dicalcium phosphate	14.00	14.50	15.00
Vitamin-trace mineral	2.50	2.50	2.50
premix*	200	200	200
Celite	20.00	20.00	20.00
Nutrient specifications			
Metabolisable energy	12.97	12.97	12.97
(MJ/kg)			
Starch	319.5	365-1	410.7
Protein	220.0	214.3	208.6
Fat	87.3	73.7	60.1
Fibre	38.2	37.8	37.4
Ca	7.67	7.68	7.68
Total P	7.24	7.06	6.87
Available P	3.45	3.45	3.45
Phytate P	3.36	3.16	2.96
Na	1.70	1.70	1.70
Digestible Lys	11.50	11.66	11.81
Digestible Met	4.98	5.30	5.62
Digestible Thr	7.59	7.70	7.80
Digestible Trp	2.37	2.44	2.51
Digestible Ile	8.20	8.45	8.70
Digestible Arg	12.93	13.38	13.83

\* The vitamin-mineral premix supplied per tonne of feed: (MIU): retinol, 12, cholecalciferol, 5; (g) tocopherol, 50; menadione, 3; thiamine, 3; riboflavin, 9; pyridoxine, 5; cobalamin, 0.025; niacin, 50; pantothenate, 18; folate, 2; biotin, 0.2; Cu, 20; Fe, 40; Mn, 110; Co, 0.25; iodine, 1, Mo, 2; Zn, 90; Se, 0.3.

Protein was derived from soyabean and rapeseed meals, maize and limited quantities of crystalline lysine, methionine and threonine in the 'slow' 220-0 g/kg protein foundation diet. The 'rapid' 208-6 g/kg protein summit diet contained considerably less soyabean meal, which was replaced by 50 g/kg casein and a total of 11-75 g/kg crystalline amino acids (arginine, isoleucine, lysine, methionine, threonine, tryptophan) so that digestible amino acid levels in the dietary treatments were comparable. An equal blend of the two diets (214-3 g/kg protein) was used as an intermediate dietary treatment so that any linear effects could be identified (Table 2).

Maize and soyabean meal were ground through a 3.2-mm hammer-mill screen before being mixed into the diets. The experimental diets were steam-pelleted at a conditioning temperature of 80°C (14s residence time) and crumbled after passing through a vertical cooler.

#### Bird management

A total of 144 d-old, male Ross 308 chicks were procured from a commercial hatchery, housed in an environmentally controlled facility and were initially offered a proprietary starter ration. At 7 d post-hatch, birds were individually identified (wing-tags), weighed and allocated into twenty-four cages on the basis of body weights so that the means and standard deviation in each cage was almost identical. Each of the three dietary treatments was then offered to eight replicate cages (six birds per cage) from 7 to 28d post-hatch. Birds had unlimited access to feed and water during the experimental feeding period under a '16-h-on' lighting regimen, and room temperature was gradually reduced from 32°C at day 1 to 22°C at day 28. Body weights were again determined at 28 d post-hatch and feed intakes for each cage were recorded over the 21-d period feeding period to calculate FCR. These calculations were adjusted by the body weight of any dead or culled birds, which were monitored on a daily basis. Total excreta collection to determine parameters of

 Table 2. Analysed nutrient specifications of foundation, intermediate and summit experimental diets

Items (g/kg)	Foundation diet	Intermediate diet	Summit diet
Starch	320.6	339.8	342.8
Protein (N)	207.6	204.5	207.0
Total amino acids	199.9	193.6	187·8
Arg	13.2	13.6	13.5
His	6.2	6.0	5.9
lle	9.3	9.5	9.3
Leu	17.9	17.6	17·0
Lys	11.9	11.8	11.6
Met	3.6	3.8	5.0
Phe	10.4	9.7	9.0
Thr	8.7	8.8	8.5
Val	10.7	10.6	10.3
Ala	10.2	9.5	8.9
Aspartic acid	20.3	17.5	15·2
Glutamic acid	39.3	38.3	36.8
Gly	9.6	8.4	7.4
Pro	12.5	13.6	14.4
Ser	10.8	10.0	9.5
Tyr	5.3	5.0	5.4

nutrient utilisation was completed over a 48-h period from 25 d post-hatch. At 28 d post-hatch, the birds were euthanised (intravenous injection of sodium pentobarbitone) in order to collect various samples for analyses.

#### Sample collection and chemical analysis

Feed intake was monitored and total excreta collected from each cage over a 48-h period to determine apparent metabolisable energy (AME), metabolisable energy:gross energy (ME:GE) ratios, N retention and N-corrected AME (AMEn). Excreta were dried in a forced-air oven at 80°C for 24 h and the GE of excreta and diets were determined using an adiabatic bomb calorimeter. The AME values of the diets on a DM basis were calculated from the following equation:

 $AME_{diet} (MJ / kg DM) = \frac{(feed intake \times GE_{diet}) - (excreta output \times GE_{excreta})}{feed intake}.$ 

ME:GE ratios were calculated by dividing AME by the GE of the appropriate diets. N contents of diets and excreta were determined using a N determinator (Leco Corporation) and N retentions calculated from the following equation:

$$N \text{ retention } \% = \frac{(\text{feed intake} \times N_{\text{diet}}) - (\text{excreta output} \times N_{\text{excreta}})}{(\text{feed intake} \times N_{\text{diet}})} \times 100.$$

AMEn (MJ/kg DM) values were calculated by correcting N retention to 0 using the factor of 36.54 kJ/g N retained in the body<sup>(12)</sup>. At day 28 following euthanasia, the abdominal cavities of the

birds were opened and digesta were collected in their entirety from the proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI) and pooled for each cage. The segments were demarcated by the mid-points between the end of the duodenal loop, Meckel's diverticulum and the ileo-caecal junction. Digesta samples were freeze-dried to determine apparent digestibilities of starch, crude protein (N) and amino acids, using  $Celite^{TM}$ (World Minerals Inc.), a source of acid insoluble ash (AIA), as the inert dietary marker. Starch concentrations in diets and digesta were determined by a procedure based on dimethyl sulphoxide,  $\alpha$ -amylase and amyloglucosidase as described by Mahasukhonthachat et al.<sup>(13)</sup>. N content was obtained using an FP-428 determinator (Leco Corporation) and AIA concentrations were determined by the method of Siriwan et al.<sup>(14)</sup>. Amino acid concentrations of diets and digesta were determined following 24-h liquid hydrolysis at 110°C in 6 M HCl and then sixteen amino acids are analysed using the Waters AccQTag Ultra chemistry (Waters) on a Waters Acquity UPLC. Tryptophan and cysteine cannot be analysed accurately by this procedure. The apparent digestibility coefficients for starch and protein (N) in four small intestinal sites and the apparent digestibility coefficients of sixteen amino acids in the three posterior small intestinal segments (insufficient digesta in PJ) were calculated from the following equation:

Apparent digestibility coefficient =  $\frac{(nutrient / AIA)_{diet}) - (nutrient / AIA)_{digesta}}{(nutrient / AIA)_{diet}}$ 

Three birds at random were selected from each cage housing birds offered the foundation and summit diets and blood samples were taken from the brachial vein before euthanasia and the anterior mesenteric vein from the same three birds following euthanasia. Blood samples were then centrifuged and the decanted plasma samples were then kept at −80°C before analysis. Concentrations of twenty proteinogenic amino acids in plasma taken from the brachial and anterior mesenteric veins were determined using precolumn derivatisation amino acid analysis with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC; Waters<sup>TM</sup> AccQTag Ultra; www.waters.com) followed by separation of the derivatives and quantification by reversed phase ultra-performance liquid chromatography<sup>(15)</sup>. All amino acids were detected by UV absorbance. This procedure is fully described in Selle *et al.*<sup>(11)</sup>.

# Statistical analysis

The experimental data derived from eight replicate cages for each of three dietary treatments were analysed as a one-way ANOVA using the IBM<sup>®</sup> SPSS<sup>®</sup> Statistics 20 program (IBM Corporation). Each replicate cage constituted an experimental unit and P < 0.05 was considered to be statistically significant. Given a P value of <0.05 from the ANOVA, Fisher's least significant difference (LSD) was calculated to compare mean values using the following equation:

$$\text{LSD } (0.05) = \frac{t\sqrt{\text{EMS} \times 2}}{n}.$$

Digestibility coefficients and disappearance rates for protein (N) and starch were determined in four small intestinal segments, but amino acid digestibility coefficients and disappearance rates were determined in three segments. Plasma concentrations of amino acids in the portal and systemic circulations in birds offered the foundation and summit diets were analysed as a 2 (foundation or summit diets)×2 (portal or systemic blood samples) factorial array of treatments. Pearson's correlations, linear and quadratic regression equations were established for selected parameters where justified. The feeding study was conducted so as to comply with specific guidelines approved by the Animal Ethics Committee of the University of Sydney.

#### Results

The effects of dietary treatments on growth performance and parameters of nutrient utilisation are shown in Table 3. There were no significant treatment effects on growth performance from one-way ANOVAs; however, the summit diet supported a numerical increase in weight gain relative to the foundation diet by 4·48% (1610 *v*. 1541 g/bird) and the linear effect closely approached significance ( $r \ 0.404$ ; P=0.0501). The acceptable mortality/cull rate of 2·78% was not related to treatment (P > 0.75). Parameters of nutrient utilisation were significantly influenced (P < 0.025- <0.001) by treatment. The summit diet supported superior nutrient utilisation in comparison with the foundation diet by 0·35 MJ (12·47 *v*. 12·12 MJ/kg) in AME, by 6·81% (0.769 *v*. 0.720) in ME:GE ratios, by 4·32 percentage units

https://doi.org/10.1017/S0007114517003257 Published online by Cambridge University Press

Table 3. Effects of dietary treatments on growth performance from 7 to 28 d and nutrient utilisation at 25-27 d post-hatch

		Growth per	rformance			Nutrient	utilisation	
Dietary treatments	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality/culls (%)	AME (MJ/kg DM)	ME:GE ratio	N retention (%)	AMEn (MJ/kg DM)
Foundation	1541	2273	1.476	4·17	12·12 <sup>a</sup>	0.720 <sup>a</sup>	60.76 <sup>a</sup>	11.09 <sup>a</sup>
Intermediate	1607	2309	1.438	2.08	12·38 <sup>b</sup>	0.747 <sup>b</sup>	64·11 <sup>b</sup>	11.31 <sup>a,b</sup>
Summit	1610	2335	1.450	2.08	12·47 <sup>b</sup>	0.769 <sup>b</sup>	65.08 <sup>b</sup>	11·41 <sup>b</sup>
SEM	23.3	26.8	0.0122	2.317	0.073	0.0035	0.525	0.075
Significance (P)	0.084	0.287	0.062	0.767	0.008	<0.001	<0.001	0.022
LSD (P<0.05)	_	_	_	_	0.215	0.0191	1.543	0.220
Linear effect								
r	0.404	0.333	-0.314	_	0.583	0.701	0.762	0.540
Р	0.0501	0.112	0.135	_	0.003	<0.001	<0.001	0.006

FCR, feed conversion ratios; AME, apparent metabolisable energy; AMEn, N-corrected AME; ME:GE, metabolisable energy:gross energy ratios; LSD, least significant difference.

<sup>a,b</sup> Mean values within column with unlike superscript letters were significantly different (P<0.05)

**Table 4.** Effects of dietary treatments on apparent digestibility coefficients and apparent disappearance rates (g/bird per d) of protein (N) and starch in four small intestinal segments (proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI), distal ileum (DI)) at 28 d post-hatch

	P	rotein (N) dige	stibility coeffici	ent	F	Protein (N) disa	appearance rat	te
Dietary treatments	PJ	DJ	PI	DI	PJ	DJ	PI	DI
Foundation	0.560	0.670 <sup>a</sup>	0.735 <sup>a</sup>	0.769 <sup>a</sup>	12.52	15.05 <sup>a</sup>	16.51 <sup>a</sup>	17·29 <sup>a</sup>
Intermediate	0.551	0.660 <sup>a</sup>	0·751 <sup>b</sup>	0.782 <sup>a</sup>	12.37	14.83 <sup>a</sup>	16·87 <sup>a</sup>	17.59 <sup>a</sup>
Summit	0.630	0.740 <sup>b</sup>	0.798 <sup>c</sup>	0.816 <sup>b</sup>	13.75	16·51 <sup>b</sup>	17·96 <sup>b</sup>	18·41 <sup>b</sup>
SEM	0.0274	0.0090	0.0049	0.0053	0.6378	0.1987	0.1827	0.2168
Significance (P)	0.126	<0.001	<0.001	<0.001	0.272	<0.001	<0.001	0.004
LSD (P<0.05)	_	0.0264	0.0145	0.0155	_	0.5838	0.5366	0.6393
Linear effect								
r	0.351	0.666	0.863	0.789	0.284	0.653	0.756	0.615
Р	0.101	<0.001	<0.001	<0.001	0.189	<0.001	<0.001	0.001
		Starch digesti	bility coefficier	ıt		Starch disap	pearance rate	
Foundation	0.664	0.808	0.910 <sup>a</sup>	0.933 <sup>a</sup>	23.04 <sup>a</sup>	28.30 <sup>a</sup>	31.71 <sup>a</sup>	32·46 <sup>a</sup>
Intermediate	0.686	0.799	0.926 <sup>a</sup>	0·954 <sup>b</sup>	25.63 <sup>b</sup>	30·10 <sup>b</sup>	34·70 <sup>b</sup>	35-69 <sup>b</sup>
Summit	0.703	0.844	0·946 <sup>b</sup>	0.962 <sup>b</sup>	26.78 <sup>b</sup>	32.38°	36·14 <sup>c</sup>	36·72 <sup>b</sup>
SEM	00 223	0.0162	0.0056	0.0059	0.8859	0.5442	0.4182	0.4524
Significance (P)	0.527	0.143	<0.001	0.006	0.025	<0.001	<0.001	<0.001
LSD (P<0.05)	_	-	0.0164	0.0173	2.602	1.598	1.228	1.329
Linear effect								
r	0.249	0.312	0.710	0.600	0.542	0.756	0.841	0.800
Р	0.252	0.139	<0.001	0.002	0.008	<0.001	<0.001	<0.001

LSD, least significant difference.

<sup>a,b,c</sup> Mean values within column with unlike superscript letters were significantly different (P<0.05).

(65.08 *v*. 60.76%) in N retention and by 0.32 MJ (11.41 *v*. 11.09 MJ/kg) in AMEn. The transition from the foundation to summit diet linearly increased (P < 0.01) all four nutrient utilisation parameters, where the impacts on ME:GE ratios (r 0.701; P < 0.001) and N retention (r 0.762; P < 0.001) were the most pronounced.

Table 4 shows the effects of dietary treatments on protein (N) and starch digestibility coefficients and disappearance rates in four small intestinal segments. The change from foundation to summit diets increased (P < 0.001) protein (N) digestibility coefficients in the three posterior small intestinal segments by 10-4% (0.740 v. 0.670) in DJ, 8.57% (0.798 v. 0.735) in PI and 6.11% (0.816 v. 0.769) in DI. Similarly, the dietary transition accelerated (P < 0.001) protein (N) disappearance rates by 9.70% (16.51 v. 15.05 g/bird per d) in DJ, 8.78% (17.96 v. 16.51 g/bird per d) in PI

and 6.48% (18.41 v. 17.29 g/bird per d) in DI. The linear effects of diet type were highly significant for both protein (N) parameters in the three posterior small intestinal segments. The transition from the foundation to summit diets significantly increased starch digestibility coefficients by 3.96% (0.946 v. 0.910) in PI and by 3.11% (0.962 v. 0.933) in DI. Dietary treatments significantly influenced starch disappearance rates in all four segments; for example, the summit diet accelerated proximal ileal starch disappearance rates by 8.78% (17.96 v. 16.51 g/bird per d). The linear effects of diet type were highly significant for starch disappearance rates in PI and DI and for starch disappearance rates in all four small intestinal segments.

The effects of three dietary treatments on apparent digestibility coefficients of essential amino acids in three small intestinal segments are shown in Table 5. The transition from the

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Table 5. Effects of four dietary treatments on apparent digestibility coefficients of essential amino acids in three small intestinal segments (distal jejurum (DJ), proximal ileum (DI)) at 28 d posthatch

	ō	0.806ª 0.850 <sup>b</sup> 0.0522 0.0151 0.0151	0.698 <0.001		2.500	ve dynar	
Lys	Ы	0.793 <sup>a</sup> 0.840 <sup>b</sup> 0.843 <sup>b</sup> 0.0069 0.02	0.704 <0.001				
	DJ	0.798 <sup>a</sup> 0.805 <sup>a</sup> 0.840 <sup>b</sup> 0.0092 0.007 0.0267	0.555 <0.001				
	DI	0.803 <sup>a</sup> 0.863 <sup>b</sup> 0.0860 <sup>b</sup> 0.0044 0.0130	0.761 <0.001		0.770 <sup>a</sup> 0.831 <sup>c</sup> 0.817 <sup>b</sup> 0.0048 <0.001	0.0141 0.667 <0.001	
Leu	Ы	0.770 <sup>a</sup> 0.822 <sup>b</sup> 0.0828 <sup>b</sup> 0.0068 0.0199	0.700 <0.001	Val	0.741 <sup>ª</sup> 0.794 <sup>b</sup> 0.790 <sup>b</sup> 0.0066	0.0193 0.628 <0.001	
	Ŋ	0.749 <sup>a</sup> 0.740 <sup>a</sup> 0.797 <sup>b</sup> 0.0085 <0.001 0.0250	0.491 0.004		0.723 <sup>a</sup> 0.711 <sup>a</sup> 0.764 <sup>b</sup> 0.0083 <0.001	0.0243 0.444 0.011	
	Ō	0.786 <sup>a</sup> 0.852 <sup>b</sup> 0.842 <sup>b</sup> 0.0047 <0.001 0.0137	0.734 <0.001		0.705ª 0.786 <sup>c</sup> 0.762 <sup>b</sup> 0.0058 <0.001	0.0170 0.652 <0.001	
ell	Ы	0.754 <sup>a</sup> 0.814 <sup>b</sup> 0.817 <sup>b</sup> 0.0064 <0.001 0.0188	0.723 <0.001	Thr	0.690 <sup>a</sup> 0.756 <sup>b</sup> 0.747 <sup>b</sup> 0.0065 <0.001	0.0191 0.640 <0.001	
	Ы	0.729 <sup>a</sup> 0.729 <sup>a</sup> 0.792 <sup>b</sup> 0.0079 <0.001	0.615 <0.001		0.689 <sup>a</sup> 0.685 <sup>a</sup> 0.737 <sup>b</sup> 0.0088 <0001	0.0258 0.465 0.007	
	D	0.799 <sup>a</sup> 0.843 <sup>b</sup> 0.838 <sup>b</sup> 0.0032 <0.001 0.0095	0.605 <0.001		0.808 <sup>a</sup> 0.863 <sup>b</sup> 0.857 <sup>b</sup> 0.0041 <0.001	0.0120 0.713 <0.001	
His	Ы	0.774 <sup>a</sup> 0.814 <sup>b</sup> 0.819 <sup>b</sup> 0.0056 <0.001 0.0166	0.435 0.013	Phe	0.774 <sup>a</sup> 0.821 <sup>b</sup> 0.821 <sup>b</sup> 0.0065 <0.001	0.0192 0.630 <0.001	
	ſſ	0.755 <sup>a</sup> 0.730 <sup>a</sup> 0.794 <sup>b</sup> 0.0082 <0.001 0.0241	0.399 0.154		0.749 <sup>a</sup> 0.735 <sup>a</sup> 0.790 <sup>b</sup> 0.0076 <0.001	0.022 0.445 0.011	
	D	0.858ª 0.902b 0.001b <0.0026 <0.001	0.783 <0.001		0.929 <sup>a</sup> 0.948 <sup>b</sup> 0.963 <sup>c</sup> 0.0026	0.0076 0.752 <0.001	
Arg	Ы	0.841 <sup>a</sup> 0.879 <sup>b</sup> 0.889 <sup>b</sup> 0.0040 <0.001	0.792 <0.001	Met	0.916 <sup>a</sup> 0.931 <sup>b</sup> 0.0033 <0.001	0.0098 0.528 0.002	
	Ы	0.832 <sup>a</sup> 0.835 <sup>a</sup> 0.880 <sup>b</sup> 0.0054 <0.001 0.0160	0.688 <0.001		0.866 <sup>a</sup> 0.849 <sup>a</sup> 0.923 <sup>b</sup> 0.0094	0.0276 0.469 0.007	ifference.
	Dietary treatments	Foundation Intermediate Summit Sem Significance (P) LiSD (P<0.05)	LIIIEAII EIIECI P P		Foundation Intermediate Summit sEM Significance (P)	LSD (P<0.05) Linear effect P	LSD. least significant difference

LbU, least significant unterence. <sup>a.b.c</sup> Mean values within column with unlike superscript letters were significantly different (*P*<0.05)

Digestive dynamics of protein and amino acids

foundation to the summit diet increased the digestibility of all essential amino acids in all three segments to highly significant (P < 0.001) extents. The distal ileal digestibility coefficient of arginine was increased by 5.01%, histidine by 4.88%, isoleucine by 7.12%, leucine by 7.10%, lysine by 5.46%, methionine by 3.66%, phenylalanine by 6.06%, threonine by 8.09% and valine by 6.10%. The three diets linearly increased digestibility coefficients of all amino acids in all three segments to significant extents with the sole exception of histidine in PJ.

The effects of three dietary treatments on digestibility coefficients of non-essential amino acids in three small intestinal segments are shown in Table 6. Relative to the foundation diet, the summit diet significant increased distal ileal digestibility coefficients of alanine by 4.67%, glutamic acid by 4.40%, proline by 9.28%, serine by 3.71% and tyrosine by 7.61%. Curiously, in the same comparison, diets had no impact on glycine and significantly decreased aspartic acid by 4.56%. Dietary treatments generally linearly increased digestibility coefficients for non-essential amino acids in one or more segments to significant extents. However, this was not the case with glycine.

The effects of three dietary treatments on disappearance rates of essential amino acids in three small intestinal segments are shown in Table 7. The transition from the foundation to summit diets significantly accelerated disappearance rates of arginine, isoleucine, lysine, methionine, threonine and valine in all three segments. Alternatively, dietary treatments did not linearly influence the disappearance rates of histidine and leucine in any segment. Moreover, phenylalanine disappearance rates were linearly retarded by the transition from foundation to summit diet to subtle, but significant (P < 0.005), extents in all three segments.

Table 8 records the effects of three dietary treatments on disappearance rates of non-essential amino acids. The transition from the foundation to summit diet linearly (P < 0.001) retarded disappearance rates of alanine, aspartic acid and glycine in all three intestinal segments and retarded (P < 0.01) serine in the PJ and DI. Conversely, the same transition accelerated (P < 0.001) the disappearance of proline in all three segments. Treatment did not have any linear effects on the disappearance rates of glutamic acid and tyrosine.

Concentrations of eighteen free amino acids in plasma taken from the anterior mesenteric or brachial veins in chicks offered the foundation or summit diets are shown in Table 9. There were no significant treatment interactions. As a main effect, the summit diet significantly increased concentrations of isoleucine by 12.8% (14.9 to 16.8 µg/ml), methionine by 44.9% (10.7-15.5 µg/ml), threonine by 42.1% (57.0-81.0 µg/ml), proline by 22.9% (52.5-64.5 µg/ml) and tyrosine by 16.6% (36.2-42.2 µg/ml), relative to the foundation diet. Conversely, the same dietary change decreased concentrations of histidine by 18.8% (14.9-12.1 µg/ml), aspartate/asparagine by 10.3% (37.7-33.8µg/ml), glycine by 23.2% (59.0–45.3µg/ml) and serine by 13.0% (63.8–55.5µg/ml), to significant extents. Concentrations of the remaining nine amino acids were not significantly influenced by the change in diets. Plasma concentrations of fourteen amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, valine, alanine, cysteine, glutamate plus glutamine, glycine and

# **N**<sup>5</sup> British Journal of Nutrition

Table 6. Effects of four dietary treatments on apparent digestibility coefficients of non-essential amino acids in three small intestinal segments (distal jejunum (DJ), proximal ileum (PI), distal ileum (DI)) at 28 d posthatch

		Ala			Aspartic acid			Glutamic acid			Gly	
Dietary treatments	DJ	PI	DI	DJ	PI	DI	DJ	PI	DI	DJ	PI	DI
Foundation Intermediate Summit SEM Significance (P) LSD ( $P < 0.05$ ) Linear effect Diet type r P	0.747 <sup>a,b</sup> 0.721 <sup>a</sup> 0.774 <sup>b</sup> 0.0097 0.003 0.0285 0.232 0.201	0.768 <sup>a</sup> 0.806 <sup>b</sup> 0.804 <sup>b</sup> 0.0072 0.002 0.0212 0.502 0.003	0.792 <sup>a</sup> 0.844 <sup>b</sup> 0.829 <sup>b</sup> 0.0054 <0.001 0.0159 0.592 <0.001	0.733 <sup>b</sup> 0.700 <sup>a</sup> 0.748 <sup>b</sup> 0.0085 0.002 0.0249 0.108 0.558	0.728 <sup>a</sup> 0.769 <sup>b</sup> 0.762 <sup>b</sup> 0.0067 <0.001 0.0196 0.504 0.003	0.877° 0.805ª 0.837 <sup>b</sup> 0.0036 <0.001 0.0106 0.639 <0.001	0.797 <sup>a</sup> 0.797 <sup>a</sup> 0.837 <sup>b</sup> 0.0061 0.001 0.0178 0.565 <0.001	0.815 <sup>a</sup> 0.857 <sup>b</sup> 0.861 <sup>b</sup> 0.0002 <0.001 0.0149 0.720 <0.001	0.840 <sup>a</sup> 0.886 <sup>b</sup> 0.877 <sup>b</sup> 0.0034 <0.001 0.0101 0.697 <0.001	0.707 <sup>b</sup> 0.667 <sup>a</sup> 0.701 <sup>b</sup> 0.0086 0.009 0.0254 0.134 0.464	0.725 <sup>a</sup> 0.752 <sup>b</sup> 0.722 <sup>a</sup> 0.0066 0.006 0.0194 0.010 0.957	0.745 <sup>a</sup> 0.790 <sup>b</sup> 0.744 <sup>a</sup> 0.0045 <0.001 0.0132 0.045 0.807
		Pro			Ser			Tyr				
Foundation Intermediate Summit diet SEM Significance (P) LSD ( $P < 0.05$ ) Linear effect Diet type r P	0.712 <sup>a</sup> 0.720 <sup>a</sup> 0.772 <sup>b</sup> 0.0079 <0.001 0.0232 0.635 <0.001	0.728 <sup>a</sup> 0.797 <sup>b</sup> 0.800 <sup>b</sup> 0.0061 <0.001 0.0179 0.774 <0.001	0.754 <sup>a</sup> 0.832 <sup>b</sup> 0.824 <sup>b</sup> 0.0040 <0.001 0.0117 0.794 <0.001	0.728 <sup>b</sup> 0.703 <sup>a</sup> 0.747 <sup>b</sup> 0.0084 0.005 0.0248 0.250 0.167	0.703 <sup>a</sup> 0.768 <sup>b</sup> 0.760 <sup>b</sup> 0.0066 0.001 0.0193 0.496 0.004	0.755 <sup>a</sup> 0.807 <sup>c</sup> 0.783 <sup>b</sup> 0.0053 <0.001 0.0155 0.602 <0.001	0.706 <sup>b</sup> 0.665 <sup>a</sup> 0.762 <sup>c</sup> 0.0096 <0.001 0.0282 0.306 0.089	0.722 <sup>a</sup> 0.766 <sup>b</sup> 0.793° 0.0077 <0.001 0.0227 0.598 <0.001	0.762 <sup>a</sup> 0.8155 <sup>b</sup> 0.820 <sup>b</sup> 0.0059 <0.001 0.0173 0.653 <0.001			

LSD, least significant difference.

a,b,c Mean values within column with unlike superscript letters were significantly different (P<0.05).

Table 7. Effects of four dietary treatments on apparent disappearance rates (g/bird per d) of essential amino acids in three small intestinal segments (distal jejunum (DJ), proximal ileum (PI), distal ileum	
(DI)) at 28 d post-hatch	

		Arg			His			lle			Leu			Lys	
Dietary treatments	DJ	PI	DI	DJ	PI	DI	DJ	PI	DI	DJ	PI	DI	DJ	PI	DI
Foundation Intermediate Summit SEM Significance (P) LSD ( $P < 0.05$ ) Linear effect Diet type r P	11.9 <sup>a</sup> 12.5 <sup>b</sup> 13.2 <sup>c</sup> 0.134 <0.001 0.394 0.804 <0.001	12.1 <sup>a</sup> 13.2 <sup>b</sup> 13.3 <sup>b</sup> 0.147 <0.001 0.432 0.770 <0.001	12:3 <sup>a</sup> 13:5 <sup>b</sup> 13:5 <sup>b</sup> 0:160 <0:001 0:471 0:712 <0:001	5.1 <sup>b</sup> 4.8 <sup>a</sup> 5.2 <sup>b</sup> 0.057 <0.001 0.167 0.311 0.083	5.2 <sup>a</sup> 5.4 <sup>a</sup> 0.034 0.108 - 0.264 0.144	5.4 <sup>a</sup> 5.6 <sup>b</sup> 5.5 <sup>a,b</sup> 0.068 0.146 - 0.272 0.131	7.4 <sup>a</sup> 7.6 <sup>a</sup> 8.2 <sup>b</sup> 0.068 <0.001 0.271 0.763 <0.001	7.6 <sup>a</sup> 8.5 <sup>b</sup> 0.101 <0.001 0.297 0.761 <0.001	7.9 <sup>a</sup> 8.9 <sup>b</sup> 8.8 <sup>b</sup> 0.110 <0.001 0.323 0.719 <0.001	14.5 <sup>a</sup> 14.3 <sup>a</sup> 15.1 <sup>b</sup> 0.179 0.018 0.524 0.307 0.087	14.9 <sup>a</sup> 15.9 <sup>c</sup> 15.7 <sup>b,c</sup> 0.192 0.005 0.295 0.439 0.012	15.6 <sup>a</sup> 17.0 <sup>b</sup> 16.3 <sup>b</sup> 0.206 0.003 0.606 0.410 0.02	10.3 <sup>a</sup> 10.4 <sup>a</sup> 10.8 <sup>b</sup> 0.120 0.009 0.354 0.520 0.002	10.2 <sup>a</sup> 10.9 <sup>b</sup> 0.121 0.001 0.357 0.620 <0.001	10.4 <sup>a</sup> 11.1 <sup>b</sup> 11.0 <sup>b</sup> 0.143 0.003 0.419 0.531 0.002
		Met			Phe			Thr			Val				
Foundation Intermediate Summit Significance (P) LSD (P<0.05) Linear effect Diet type r P	3.4 <sup>a</sup> 3.6 <sup>b</sup> 5.1 <sup>c</sup> 0.0592 <0.001 0.174 0.855 <0.001	3.6 <sup>a</sup> 3.9b 5.3 <sup>c</sup> 0.0524 <0.001 0.154 0.898 <0.001	3.6 <sup>a</sup> 4.0 <sup>b</sup> 5.4 <sup>c</sup> 0.0512 <0.001 0.150 0.901 <0.001	8.4 <sup>b</sup> 7.9 <sup>a</sup> 7.9 <sup>a</sup> 0.093 <0.001 0.273 -0.599 <0.001	8.7 <sup>b</sup> 8.8 <sup>b</sup> 8.2a 0.104 0.001 0.308 -0.552 <0.001	9.1 <sup>b</sup> 9.2 <sup>b</sup> 8.6 <sup>a</sup> 0.113 <0.001 0.332 -0.528 0.002	6.5 <sup>a</sup> 6.6 <sup>a</sup> 7.0 <sup>b</sup> 0.082 0.022 0.241 0.492 0.004	6.5 <sup>a</sup> 7.3 <sup>b</sup> 7.1 <sup>b</sup> 0.082 <0.001 0.240 0.563 <0.001	6.6 <sup>a</sup> 7.6 <sup>c</sup> 7.2 <sup>b</sup> 0.081 <0.001 0.296 0.519 0.002	8.4 <sup>a</sup> 8.3 <sup>a</sup> 8.8 <sup>b</sup> 0.082 0.028 0.297 0.433 0.013	8.6 <sup>a</sup> 9.3 <sup>b</sup> 9.1 <sup>b</sup> 0.109 <0.001 0.320 0.518 0.002	8.9 <sup>a</sup> 9.7 <sup>b</sup> 9.4 <sup>b</sup> 0.125 <0.001 0.367 0.472 0.006			

LSD, least significant difference.

a,b,c Mean values within column with unlike superscript letters were significantly different (P<0.05).

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ffects of four dietary treatments on appa	um (DI)) at 28 d post-hatch

		Ala			Aspartic acid			Glutamic acid			Gly	
Dietary treatments	ΓΟ	Ы	D	DJ	Ы	DI	DJ	Ы	D	DJ	Ы	D
Ecundation	de a	a E <sup>b</sup>	0.7b	16.10	16.00	10.20	22.0	24.7	26.7 <sup>a</sup>	7.20	7 60	7.70
	ı c			- 1				2.40	1000		20	- 1
Intermediate	-G·/	8.47	ά	13.5	14.8	10.01	0.55	30.3	37:32	N.O	0.1	1.3
Summit	$7.7^{a}$	8.0 <sup>a</sup>	8·2 <sup>a</sup>	12.7 <sup>a</sup>	12.9 <sup>a</sup>	14.1 <sup>a</sup>	34.2	35.3	35.9 <sup>a</sup>	$5.8^{a}$	$5.9^{a}$	6.1 <sup>a</sup>
SEM	0.102	0.104	0.121	0.202	0.185	0.189	1.09	0.405	1.694	0.076	0.053	0.100
Significance (P)	<0.001	0.004	0.004	<0.001	<0.001	<0.001	0.454	0.059	0.043	<0.001	<0.001	<0.001
LSD(P < 0.05)	0.301	0.305	0.355	0.594	0.545	0.555	I	I	1.35	0.225	0.239	0.294
LIIIEAI EIIECI r	-0.565	-0.605	-0.539	-0.911	0.922	-0.953	0.161	0.279	0.153	208.0-	-0.926	-0.901
. d	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.379	0.122	0.404	<0.001	<0.001	<0.001
		Pro			Ser			Tyr				
Foundation diet	9.6 <sup>a</sup>	9.8 <sup>a</sup>	10.2 <sup>a</sup>	8.5°	8.5 <sup>b</sup>	8.8 <sup>b</sup>	4.0 <sup>b</sup>	4.1 <sup>a</sup>	4.4 <sup>a</sup>			
Intermediate diet	10.8 <sup>b</sup>	11.9 <sup>b</sup>	12·5 <sup>b</sup>	7.7 <sup>a</sup>	8.4 <sup>b</sup>	8:9 <sup>0</sup>	3.6 <sup>a</sup>	4.2 <sup>a</sup>	4.5 <sup>a</sup>			
Summit diet	12.4 <sup>c</sup>	12.8 <sup>c</sup>	13.2°	7.9 <sup>a,b</sup>	8.0 <sup>a</sup>	8.3 <sup>a</sup>	4.5 <sup>c</sup>	4.6 <sup>b</sup>	4.9 <sup>b</sup>			L
SEM	0.131	0.141	0.154	0.099	0.103	0.119	0.059	0.059	0.069			ηξ
Significance (P)	<0.001	<0.001	<0.001	<0.001	0.005	0.003	<0.001	<0.001	<0.001			ges
LSD ( $P < 0.05$ )	0.384	0.414	0.451	0.290	0.303	0.351	0.174	0.174	0.202			SUV
Linear effect				0470	007.0	100	0010		100 0			ve
	0.942	0.909	0.928	-0.472	-0:4:00	112.0-	0.100	0.505	107-0			a
ط	<0.001	<0.001	<0.001	<0.001	0.004	0.246	0.381	0.091	0.120			yn
I SD loset cignificant difference	forence											an 
LOD, reast signinucant universitive. <sup>a.b.c</sup> Mean values within column with unlike suberscript letters were significantly different ( <i>D</i> >0.05)	rerence. Column with unlike	a emorecript lattor	re were cignificant	thy different $(D > 0)$	05)							11C
		a auperaction retre-										s

proline) were significantly higher in the portal than systemic circulation. Plasma concentrations of the remaining four amino acids (arginine, threonine, serine, tyrosine) were numerically higher in the portal bloodstream.

### Discussion

Dangin *et al.*<sup>(1)</sup> developed the concept of 'slow' and 'fast' protein and concluded that the rate of protein digestion independently modulates postprandial protein deposition in humans. In a following study, Dangin *et al.*<sup>(2)</sup> considered that 'fast' protein may be more beneficial than a 'slow' protein to limit body protein loss in elderly subjects. Subsequently, Tang *et al.*<sup>(3)</sup> compared the effects of whey hydrolysate, casein and soya protein isolate on muscle protein synthesis in young men and proposed that the differences observed may be related to how quickly the proteins are digested. Interestingly, there were significant, negative correlations between the distal ileal disappearance rates of eight amino acids with FCR as shown in Table 10. Thus, the inference is that 'fast' protein, or the rapid disappearance/absorption of certain amino acids, benefits feed efficiency in poultry.

# Growth performance

The overall performance of male broiler chicks from 7 to 28 d post-hatch in the present study was very acceptable in comparison with 2014 Ross 308 performance objectives (shown in parentheses). The overall weight gain was 1576 g/bird (1387 g/ bird), feed intake was 2286 g/bird (2052 g/bird) and FCR was 1.451 (1.479). As expected, the transition from the foundation, via the intermediate, to the summit diet linearly accelerated protein (N) disappearance rates in the three posterior small intestinal segments by 6.48 to 9.70%. The substitution of soyabean meal in the foundation diet with casein and crystalline amino acids in the summit diet had the desired impact of generating a 'rapid protein' diet. On the basis of a pair-wise comparison, birds offered the summit diet outperformed their foundation diet counterparts by 4.48% (1610 v. 1541 g/bird; P=0.041) in weight gain. Interestingly, there was a positive association ( $r \ 0.706$ ; P < 0.001) between distal ileal protein (N) disappearance rates and weight gains (Fig. 1), which implies that 'rapid protein' was advantageous in this respect.

# Nutrient utilisation and starch digestibility

The transition from slow-protein foundation to rapid-protein summit diets had unequivocally positive effects on parameters of nutrient utilisation (Table 2). This is illustrated by the quadratic relationship between proximal ileal protein (N) disappearance rates with AME (r 0.530; P=0.032) and the linear relationship with N retention (r 0.491; P=0.015), as shown in Figs. 2 and 3. These relationships suggest that rapid protein disappearance rates enhanced energy utilisation, which may have stemmed from the positive influence the transition from slow- to rapid-protein diets had on apparent digestibly coefficients and disappearance rates of starch (Table 4). As illustrated in Fig. 4 and 5, there were linear relationships between proximal ileal protein (N) disappearance rates and proximal ileal

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Table 9. Plasma concentrations (µg/ml) of free amino acids (*n* 18) taken from the portal (anterior mesenteric vein) or systemic (brachial vein) circulation in chicks offered the foundation or summit diet

Diets	Source	Arg	His	lle	Leu	Lys	Met	Phe	Thr	Trp
Foundation	Portal	92.7	16.2	17.8	31.7	31-4	11.6	22.3	58.8	6.5
	Systemic	88.3	13.7	12.0	22.3	25.7	9.8	17.7	55.2	5.5
Summit	Portal	101.7	14.5	20.0	33.2	29.9	16.5	22.4	81.8	6.8
	Systemic	95.3	9.7	13.5	22.3	23.0	14.5	17.2	80.2	6.3
SEM		3.23	0.692	0.601	0.963	1.54	0.444	0.634	2.22	0.222
Main effects: o	diet									
Foundation		90.5	14·9 <sup>b</sup>	14·9 <sup>a</sup>	27.0	28.5	10·7 <sup>a</sup>	20.0	57.0 <sup>a</sup>	6.0
Summit		98.5	12·1 <sup>a</sup>	16⋅8 <sup>b</sup>	27.8	26.4	15.5 <sup>b</sup>	19.8	81.0 <sup>b</sup>	6.6
Source										
Portal		97.2	15·3 <sup>b</sup>	19·9 <sup>b</sup>	32·5 <sup>b</sup>	30.6 <sup>b</sup>	14·1 <sup>b</sup>	22·4 <sup>b</sup>	70.3	6.6 <sup>b</sup>
Systemic		91.8	11.7 <sup>a</sup>	12.8ª	22.3ª	24.3 <sup>a</sup>	12·2 <sup>a</sup>	17·4 <sup>a</sup>	67.7	5.9 <sup>a</sup>
Significance (	P)									
Diet (D)		0.095	0.009	0.040	0.558	0.346	<0.001	0.847	<0.001	0.074
Source (S)		0.255	0.001	<0.001	<0.001	0.009	0.006	<0.001	0.412	0.031
D×S interaction		0.826	0.247	0.671	0.588	0.797	0.844	0.721	0.745	0.449
		Val	Ala	Asp + Asx	Cys	Glu + Gln	Gly	Pro	Ser	Tyr
Foundation	Portal	29.9	114.3	39.3	14.4	222.7	63.6	56.9	64.8	38.5
	Systemic	24.0	96.7	36.0	12.2	202.0	54.3	48.0	62.8	33.8
Summit	Portal	30.0	106.5	35.9	13.9	228.0	50.7	70·1	59.3	44.2
	Systemic	22.2	92.3	31.8	11.0	206.5	40.0	59.0	51.7	40.2
SEM	-	0.862	3.747	1.19	0.446	5.696	1.966	2.292	2.332	1.791
Main effects: of	diet									
Foundation		27.0	105.5	37·7 <sup>b</sup>	13.3	212.4	59·0 <sup>b</sup>	52.5 <sup>a</sup>	63·8 <sup>b</sup>	36·2 <sup>a</sup>
Summit		26.1	99.4	33.8ª	12.5	217.3	45·3 <sup>a</sup>	64·5 <sup>b</sup>	55.5ª	42·2 <sup>b</sup>
Source										
Portal		29.9 <sup>b</sup>	110·4 <sup>b</sup>	37·6 <sup>b</sup>	14·1 <sup>b</sup>	225·4 <sup>b</sup>	57·1 <sup>b</sup>	63.5ª	62.0	41.3
Systemic		23.1ª	94.5 <sup>a</sup>	33.9 <sup>a</sup>	11.6 <sup>a</sup>	204·3 <sup>a</sup>	47·2 <sup>a</sup>	53.5 <sup>b</sup>	57.3	37.0
Significance (	P)									
Diet (D)		0.473	0.266	0.034	0.214	0.543	<0.001	0.001	0.020	0.027
Source (S)		<0.001	0.007	0.041	0.001	0.016	0.002	0.006	0.164	0.103
D×S intera	ction	0.449	0.747	0.841	0.576	0.953	0.806	0.744	0.403	0.904

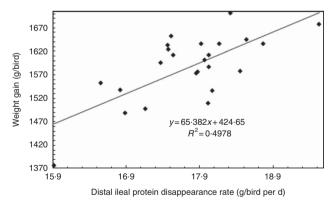
<sup>a,b</sup> Mean values within column with unlike superscript letters were significantly different (P<0.05).

Table 10. Pearson' correlations between distal ileal disappearance rate	s
(g/bird per d) of sixteen amino acids and feed conversion ratios (ranked	I)

Amino acids	Correlation coefficient (r)	Significance (P)		
lle	-0.513	0.010		
Thr	-0.502	0.012		
Lys	-0.502	0.012		
Val	-0.491	0.015		
Arg	-0.485	0.016		
Leu	-0.46	0.024		
Pro	-0.426	0.038		
His	-0.412	0.046		
Glutamic acid	-0.374	0.072		
Tyr	-0.248	0.244		
Met	-0.177	0.409		
Phe	-0.176	0.411		
Ser	-0.125	0.561		
Ala	-0.118	0.583		
Gly	0.106	0.621		
Aspartic acid	0.359	0.085		

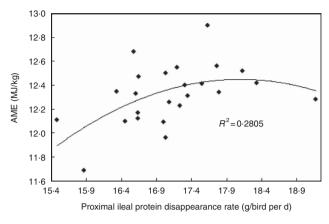
starch digestibility coefficients (r 0.638; P < 0.001) and proximal ileal starch disappearance rates (r 0.876; P < 0.001).

The importance of biophysical and biochemical starchprotein interactions on energy utilisation in poultry is accepted, although these interactions have yet to be described precisely<sup>(16)</sup>. The relevant Rooney and Pflugfelder<sup>(17)</sup> review



**Fig. 1.** Linear relationship between distal ileal protein (N) disappearance rates with 7 to 28 d weight gain (r 0.706; P < 0.001).

focuses on starch–protein interactions involving starch granules and prolamin proteins in the endosperm of feed grains. The dietary starch was mainly derived from maize in this study, and the interactions with protein from either casein or soyabean meal, rather than maize *per se*, appear to be influential. There is the possibility that negative interactions between maize starch and soya protein are being attenuated by the partial replacement of soyabean meal with casein. Soya protein has been shown to interact with wheat starch under *in vitro* conditions<sup>(18)</sup>.



**Fig. 2.** Quadratic relationship between proximal ileal protein (N) disappearance rates with apparent metabolisable energy (AME) (r 0.530; P = 0.032).

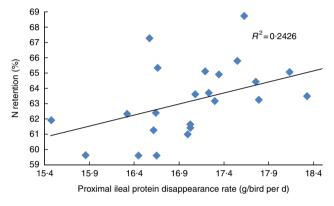


Fig. 3. Linear relationship between proximal ileal protein (N) disappearance rates with N retention (r 0.491; P = 0.015).

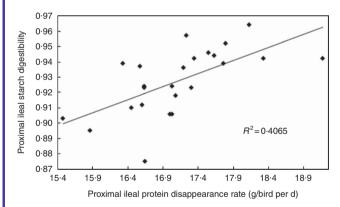


Fig. 4. Linear relationship between proximal ileal protein (N) disappearance rates and proximal ileal starch digestibility coefficients (r 0.638; P = 0.001).

Similar starch–protein interactions presumably could take place during steam-pelleting of the diets and/or in the avian digestive tract. Importantly, there is a precedent for different protein sources impacting on energy utilisation and starch digestibility. Sydenham *et al.*<sup>(19)</sup> reported such outcomes following the partial substitution of soyabean meal with fishmeal in broiler diets. This substitution increased AME by 0.74 MJ (14.47 *v.* 13.73 MJ/kg; P < 0.005) and ME:GE ratios by 8.03% (0.767 *v.* 0.710; P < 0.001). The same substitution significantly increased starch digestibility

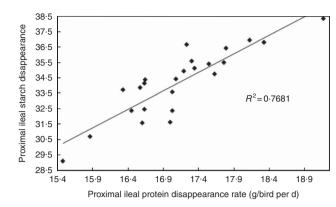


Fig. 5. Linear relationship between proximal ileal protein (N) disappearance rates and and proximal ileal starch disappearance rates (*r* 0.876; *P*<0.001).

coefficients in four small intestinal segments culminating in an increase of  $18\cdot1\%$  (0.926 *v*. 0.784; *P*<0.001) in the DI.

Starch-protein interactions in wheat can influence glycaemic responses in humans<sup>(20)</sup> or intestinal uptakes of glucose. Intestinal uptakes of amino acids and sugars are complex and interactive, as reviewed by Stevens et  $al^{(21)}$  and amino acids and glucose may effectively compete for absorption along the small intestine<sup>(22,23)</sup>. Certainly, Vinardell<sup>(24)</sup> concluded that intestinal uptakes of amino acids and sugars are subject to mutual inhibition, which may particularly apply to competition for co-absorption with Na via their respective Na<sup>+</sup>-dependent transport systems. Perhaps a better comprehension of starchprotein interactions would be gained by focusing on their constituent sugars and amino acids. Starch pasting profiles of feed grains determined by rapid visco-analysis (RVA) may be indicative of their nutritive value in poultry<sup>(25)</sup>; therefore, it is interesting that amino acids have been shown to influence RVA starch pasting profiles in several studies including Ito et al.<sup>(26)</sup>. The starch-protein interaction is an intriguing target for future research as one speculative interpretation is that the dietary provision of 'rapid protein' may have favourably manipulated the competition between glucose and amino acids for intestinal uptakes both in the Sydenham et al.<sup>(19)</sup> study and the present study.

# Apparent protein and amino acid digestibility

Apparent protein (N) digestibility coefficients in the three caudal small intestinal segments of the birds offered the summit diet were significantly higher than their foundation diet counterparts, and this translated to significant linear increases in protein (N) disappearance rates in the corresponding segments. The improvements in apparent protein (N) digestibility coefficients may be attributed partially to the inherently better digestibility of casein and crystalline amino acids in comparison with soya protein. From data generated by Bryden *et al.*<sup>(27)</sup>, the transition from summit to foundation diets would improve ileal protein digestibility by an estimated 3.72% (0.837 *v.* 0.807). Thus, the 6.11% improvement observed in the present study is somewhat greater than could be expected from the manipulation of the diets.

The transition from foundation to summit diets linearly increased (P < 0.001) the average apparent digestibility coefficients of the total of 16 amino acids by 5.42% (0.797 v. 0.756) in the DJ, by

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6.36% (0.819 *v*. 0.770) in the PI and by 5.53% (0.839 *v*. 0.795) in the DI. The average apparent jejunal digestibility of eleven proteinbound amino acids (0.771) in the summit diet was surpassed by the average apparent digestibility of five amino acids (0.834) that were present in the diet as both crystalline and protein-bound amino acids by 8.17%. This advantage diminished to 6.13 and 4.60% in the proximal and DI, respectively. These outcomes reflect the accepted view that crystalline amino acids are rapidly 'digested' and absorbed in the anterior small intestine and are fully bio-available in poultry<sup>(28)</sup>.

Threonine and proline are two of the most abundant amino acids in porcine mucin<sup>(29)</sup> and the transition from foundation to summit diets improved the apparent distal ileal digestibility of these amino acids by 8.09 and 9.28%, respectively. Given that the average improvement for the balance of fourteen amino acids was 4.36%, there is the implication that increasing dietary casein and crystalline synthetic amino acids reduced mucin secretion and endogenous amino acid flows, thereby enhancing apparent amino acid digestibility coefficients.

#### Amino acid disappearance rates

Essentially, the transition from the foundation to summit diet linearly accelerated amino acid disappearance rates in three small intestinal segments (Tables 7 and 8). The effects of the dietary transition were highly significant (P < 0.001) in all three segments for three essential amino acids - arginine (32.6%), isoleucine (12.9%) and methionine (34.1%) – which were present in the summit diet as both crystalline (proportions shown in parentheses) and protein-bound amino acids. The effects of the dietary transition were highly significant (P < 0.001) in all three segments for four non-essential amino acids (alanine, aspartic acid, glycine and proline). Conversely, significant linear effects were not observed for histidine, glutamic acid and tyrosine in any segment. This is the case as following the transition from foundation to summit diet there is a highly significant linear relationship ( $r \ 0.822$ ; P < 0.001) between percentage increases in distal ileal amino acid disappearance rates and percentage increases in free amino acid concentrations in blood taken from the anterior mesenteric vein.

# Concentrations of amino acids in portal systemic circulations

Intestinal uptakes of nutrients, including amino acids, are crucial to bird performance<sup>(9)</sup>; nevertheless, absorbed amino acids do not necessarily enter the portal circulation to become available for protein accretion. As reviewed by Wu et al.<sup>(30)</sup>, this subject is complex because a large proportion of absorbed amino acids become involved in anabolic and catabolic pathways in enterocytes. Moreover, amino acids that do enter the portal circulation may have entered the gut mucosa from either the gut lumen or the arterial circulation and are not necessarily amino acids of dietary origin. Amino acids in enterocytes may be synthesised into proteins to maintain gut integrity or serve as precursors for digestive enzymes, mucin, nucleotides, polyamines and amino acids<sup>(10)</sup>. In addition, amino acids are subject to catabolism in the gut mucosa, and Reeds et al.<sup>(31)</sup> concluded that amino acids are critical energy sources for the intestinal mucosa, with the caveat that whether they are subject to nutritional regulation requires further investigation. Either glucose or amino acids, especially glutamate/glutamine, are catabolised in avian enterocytes for the provision of energy<sup>(32)</sup>. Glucose and glutamine provide energy to the gut mucosa in rats to approximately equal extents, but it appears that energy is derived more efficiently from glucose<sup>(33)</sup>. Thus, there is a 'catabolic ratio' between amino acids and glucose within enterocytes and it should be advantageous to manipulate this ratio so that more glucose and less amino acids are catabolised. If achieved, amino acids would be spared from catabolism to enter the portal circulation and become available for protein accretion, and the copious energy requirement of the gut would be met more efficiently.

This is the rationale for the determination of concentrations of free amino acids in the portal and systemic blood samples (Table 9). Overall, the sum concentrations of eighteen amino acids tabulated was 929.5 µg/ml in the portal circulation, which declined by 12.7 % to 811.0 µg/ml in the systemic circulation. All amino acids declined, to significant or numerical extents, which is indicative of their utilisation in the body. The four largest relative reductions were observed for the branched-chain amino acids (isoleucine 35.7%, leucine 31.4%, valine 22.7%) and histidine (23.5%). As a main effect, the change from the 'slow' summit diet to the 'rapid' protein diet significantly increased plasma concentrations of isoleucine, methionine, threonine, proline and tyrosine but significantly decreased concentrations of histidine, aspartate/asparagine, glycine and serine. It is probably relevant that isoleucine, methionine and threonine were present as crystalline amino acids at higher inclusions in the summit diet.

A direct comparison of the impact of the 'slow' summit diet in comparison with the 'rapid' protein foundation diet on amino acid concentrations in the portal circulation per se is of interest. Of relevance, is that crystalline amino acids represented 8.8% of lysine, 30.8% of methionine and 1.9% of threonine of total dietary amino acids in the foundation diet. In contrast, in the summit diet, crystalline amino acids comprised 14.3% of total lysine, 34.1% methionine, 12.3% threonine, 18.6% tryptophan, 32.6% arginine and 12.9% isoleucine. Birds offered the summit diet had significantly increased concentrations of methionine by 42.2% (16.5 v. 11.6 µg/ml; P<0.001), threonine by 39.1% $(81.8 v. 58.8 \mu g/ml; P < 0.001)$  and proline by 23.2% (70.1 v.  $56.9 \,\mu\text{g/ml}; P = 0.01$ ) relative to their counterparts on foundation diet on the basis of pair-wise comparisons. It should be noted that methionine and threonine are two of the first three limiting amino acids in poultry diets. On the other hand, glycine concentrations were decreased by 20.3% (P=0.004) and the balance of amino acids were not influenced (P > 0.125) by the transition from foundation to summit diets. Six amino acids were present in the summit diet in both crystalline and proteinbound forms, and the transition from slow to rapid protein diets increased their collective concentrations in the portal circulation by 17.4% from 219 to 257 µg/ml. In contrast, there were twelve protein-bound amino acids in the summit diet, and the transition fractionally decreased their concentrations by 0.84% (709 v. 715 µg/ml). Although not conclusive, this outcome appears to be consistent with the possibility that crystalline amino acids are less prone to be catabolised in the gut mucosa

than their protein-bound counterparts. The assertion here is that because crystalline amino acids are rapidly absorbed in the anterior small intestine, where more glucose is available as an alternative energy substrate, they may be spared from catabolism. Thus, this preliminary investigation into free amino acid plasma concentrations in poultry suggests that accelerating protein digestion rates may be a dietary means of manipulating the catabolic ratio between amino acids and glucose. Reductions in ammonia concentrations in the portal circulation could provide more specific indications of reductions in amino acid catabolism. Stoll et al.<sup>(34)</sup> reported that net portal outflows of ammonia accounted for 18% of total amino acid nitrogen intake in pigs, and thus any dietary strategy that reduces concentrations of ammonia in the anterior mesenteric vein has probably suppressed amino acid catabolism in the gut mucosa. This will be the subject of future investigations.

#### Summary

In the present study, as defined, 'rapid protein' advantaged weight gain and parameters of nutrient utilisation and influenced post-enteral availability of amino acids in broiler chickens. The dietary provision of 'rapid protein' increased starch digestibility coefficients in both ileal segments and starch disappearance rates in four small intestinal segments, which appeared to be associated with linear increases in parameters of nutrient utilisation (AME, ME:GE ratios, N retention, AMEn). In addition, the present study provided indications that plasma concentrations of free amino acids in the portal circulation can be influenced by dietary manipulation. These outcomes indicate that future research into the relevance of protein and starch digestive dynamics to chicken-meat production is justified.

# Acknowledgements

The authors would like to acknowledge Professor Mingan Choct and the Poultry CRC for their support of both Dr Ha Truong's PhD candidature and this particular study. The authors would also like to thank the indefatigable Ms Joy Gill and her team in the Poultry Research Foundation, and Mr Bernie McInerney, Dr Leon McQuade and their team at Australian Proteome Analytical Facility (Macquarie University) for their invaluable contributions. The funders had no role in the design, analysis or writing of

this article. The authors' contributions were as follows: H. H. T. contributed to the study design and data analyses; P. V. C. contributed to the study design, diet formulation and manuscript writing; H. H. T.

and A. F. M contributed to data collection; H. H. T., A. F. M, P. H. S. and S. Y. L. contributed to manuscript writing, P. H. S. and S. Y. L. contributed to experimental design and data analysis. All the authors read and approved the final version of the manuscript.

The authors declare that there are no conflicts of interest.

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