

The influence of substituting dietary peptide-bound with free amino acids on nitrogen metabolism and acid-base balance of broiler chickens depends on asparagine and glutamine supply

Supplemental file

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Supplementary Table 1. Precaecal crude protein and amino acid digestibility (%) of the diets in the digestibility trial.

	Basal mix + soy protein isolate	Basal mix + maize starch	Pooled SEM	<i>P</i> -value
Crude protein	89 ^a	86 ^b	0.77	0.001
Alanine	89	88	0.95	0.082
Arginine	95 ^a	94 ^b	0.35	0.002
Asp+Asn [*]	89 ^a	84 ^b	1.03	0.001
Cysteine	84	84	0.62	0.972
Glu+Gln [†]	93 ^a	90 ^b	0.50	<0.001
Glycine	90	90	0.54	0.749
Histidine	90 ^a	87 ^b	0.78	<0.001
Isoleucine	92 ^a	90 ^b	0.52	0.002
Leucine	92	91	0.72	0.093
Lysine	94 ^a	93 ^b	0.51	0.046
Methionine	95	96	0.35	0.065
Phenylalanine	93 ^a	91 ^b	0.80	0.016
Proline	91 ^a	89 ^b	0.56	0.001
Serine	86 ^a	80 ^b	0.72	<0.001
Threonine	86 ^a	84 ^b	0.99	0.007
Tryptophan	85	85	0.91	0.975
Tyrosine	91 ^a	90 ^b	0.75	0.016
Valine	93	92	0.45	0.355

SEM, standard error of the mean.

^{a,b}Different letters indicate significant differences ($P<0.050$)

^{*}Aspartic acid and Asparagine analysed together.

[†]Glutamic acid and Glutamine analysed together.

Supplementary Table 2. Analysed nutrient composition of the experimental diets (g/kg on a 88% DM basis unless otherwise stated).

Diet*	Digestibility trial		Substitution trial									Digestible AA in all diets [†]
	BM + SPI	BM + maize starch	0FAA	25FAA-Asp/Glu	25FAA-Asx/Glx	50FAA-Asp/Glu	50FAA-Asx/Glx	75FAA-Asp/Glu	75FAA-Asx/Glx	100FAA-Asp/Glu	100FAA-Asx/Glx	
DM (g/kg)	911	908	917	921	923	924	922	922	921	925	921	-
Crude protein	168	103	167	164	166	160	164	157	162	154	160	-
Crude fat	n.a.	n.a.	57.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-
Crude fibre	n.a.	n.a.	13.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-
Crude ash	n.a.	n.a.	40.0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-
Starch	n.a.	n.a.	559	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-
Titanium dioxide	4.8	4.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-
Calcium	9.0	9.2	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	-
Phosphorus	7.8	7.3	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	-
ME _N [‡] (MJ/kg on a 88% DM basis)	n.c.	n.c.	14.0	13.9	14.0	13.9	13.9	13.8	13.9	13.8	13.9	-
Sodium	n.a.	n.a.	3.1	2.7	2.7	2.4	2.4	2.1	2.1	1.8	1.9	-
Potassium	n.a.	n.a.	2.5	2.3	2.3	2.2	2.2	2.2	2.2	2.1	2.1	-
Chloride	n.a.	n.a.	2.0	2.2	2.2	2.1	2.3	2.6	2.5	2.7	2.7	-
DEB [§] (mEq/kgTM)	-	-	161	132	131	115	111	86	90	66	67	-
Alanine	8.3	5.5	8.7	8.4	8.4	8.4	8.4	8.4	8.3	8.4	8.5	7.4
Arginine	12.0	7.0	12.8	12.2	12.4	12.2	12.1	12.2	12.0	12.0	12.3	11.4
Asp+Asn	14.3	6.3	15.2	14.4	14.8	14.6	14.6	14.6	14.3	14.4	14.7	12.8
Cysteine	2.8	2.1	2.9	2.8	2.8	2.7	2.7	2.7	2.6	2.6	2.6	2.4
Glu+Gln	31.3	18.4	33.0	31.6	32.0	32.0	32.2	32.3	31.7	32.2	32.7	29.3
Glycine	8.4	5.8	9.0	8.6	8.7	8.6	8.6	8.5	8.4	8.4	8.6	7.6
Gly _{equi}	14.4	9.2	15.3	14.6	14.8	14.6	14.7	14.4	14.3	14.4	14.6	12.8
Histidine	4.5	2.6	4.7	4.5	4.6	4.6	4.6	4.7	4.7	4.7	4.8	4.0
Isoleucine	8.4	5.1	8.6	8.1	8.3	8.2	8.2	8.2	7.9	8.1	8.2	7.7
Leucine	15.3	10.0	16.5	15.8	15.9	16.0	16.1	16.1	15.7	15.9	16.1	14.2
Lysine	11.2	7.0	11.9	11.4	11.6	11.4	11.5	11.6	11.4	11.5	11.7	10.5
Methionine	5.5	4.6	5.8	5.5	5.6	5.6	5.6	5.6	5.5	5.5	5.6	5.2

Table continued on next page.

Supplementary Table 2. Continuation.

Diet*	Digestibility trial		Growth trial									Digestible AA in all diets [†]
	BM + SPI	BM + maize starch	0FAA	25FAA- Asp/Glu	25FAA- Asx/Glx	50FAA- Asp/Glu	50FAA- Asx/Glx	75FAA- Asp/Glu	75FAA- Asx/Glx	100FAA- Asp/Glu	100FAA- Asx/Glx	
Methionine+Cysteine	8.3	6.7	8.7	8.4	8.4	8.4	8.4	8.4	8.1	8.1	8.2	7.6
Phenylalanine	8.1	4.6	8.6	8.2	8.3	8.4	8.4	8.4	8.1	8.3	8.4	7.5
Proline	11.7	8.4	12.7	11.9	12.2	12.3	12.2	12.1	12.1	12.3	12.4	10.6
Serine	9.5	5.4	8.8	8.4	8.5	8.4	8.4	8.3	8.3	8.3	8.4	7.2
Threonine	8.5	5.7	8.1	7.7	7.8	7.7	7.7	7.6	7.5	7.5	7.6	6.4
Tryptophan	2.0	1.2	1.9	1.8	1.8	1.7	1.8	1.6	1.7	1.6	1.6	1.5
Tyrosine	6.8	4.3	6.2	5.9	6.0	5.9	6.0	5.9	5.7	5.7	5.8	5.5
Valine	13.1	9.6	12.1	11.5	11.8	11.6	11.6	11.6	11.5	11.6	11.7	10.6

AA, amino acids; DM, dry matter; BM, basal mix; SPI, soy protein isolate; n.a., not analysed; n.c., not calculated.

*0FAA (no free amino acid substitution): basal mix + 80 g/kg SPI; digestible amino acids in 80 g SPI/kg of the 0FAA diet were substituted by 25 (25AA), 50 (50AA), 75 (75AA), or 100 (100AA) percent with free amino acids using a mixture of all 20 proteinogenic amino acids except for asparagine, aspartic acid, glutamine, and glutamic acid; digestible asparagine+aspartic acid and glutamine+glutamic acid in SPI of the 0FAA diet were substituted by the same levels as the other amino acids, either with aspartic acid and glutamic acid (Asp/Glu), or with 50/50 mixes of asparagine/aspartic acid and glutamine/glutamic acid (Asx/Glx).

[†]Formulated digestible AA concentration based on results of the digestibility trial.

[‡]Calculated metabolisable energy (N-corrected).

[§]Dietary electrolyte balance (mEq/kgTM) = Na (mg/kg TM)/23.0 + K (mg/kg TM)/39.1 – Cl (mg/kg TM)/35.5

^{||}Glycine + 0.7143 × Serine.

Supplementary Table 3. Effects of diets and time after bleeding on blood traits of broiler chickens in the digestibility trial analysed using the i-STAT-Analyser fitted EC8+ Cartridges (n=8 individual birds/treatment).

Treatment	Basal mix + soy protein isolate		Basal mix + maize starch		Pooled SEM	ANOVA (<i>P</i> -values)*		
	1 st	2 nd	1 st	2 nd		Measurement	Treatment	Measurement × Treatment
pH	7.35	7.34	7.38	7.37	0.02	0.682	0.223	0.852
pCO ₂ (kPa)	5.9	5.9	5.4	5.5	0.3	0.701	0.037	0.866
HCO ₃ (mmol/L)	24	23	24	24	1.1	0.925	0.665	0.995
BE (mmol/L)	-2	-2	-1	-1	1.3	0.844	0.553	0.950
TCO ₂ (mmol/L)	25	25	25	25	1.1	0.951	0.667	0.951
Sodium (mmol/L)	142	141	140	140	0.7	0.656	0.019	0.458
Potassium (mmol/L)	6.3	6.2	6.0	5.7	0.2	0.222	0.034	0.621
Chloride (mmol/L)	108	108	108	108	1.0	0.762	0.559	0.927
Anion gap (mmol/L)	16	16	13	14	0.8	0.715	0.007	0.584
Glucose (mmol/L)	13	13	20	21	1.3	0.914	<0.001	0.854
Hematocrit (% PCV)	20	20	19	19	0.7	0.463	0.119	0.994
Haemoglobin (g/L)	66	68	64	65	2.4	0.397	0.126	0.900

SEM, standard error of the mean; PCO₂, carbon dioxide partial pressure; HCO₃, bicarbonate; BE, base excess; TCO₂, total carbon dioxide; kPa, kilo Pascal; PCV, packed cell volume.

*Blood traits were statistically analysed by 2-factorial ANOVA using the model $y_{ijk} = \alpha + \text{treatment}_i + \text{measurement}_j + \text{treatment}_i \times \text{measurement}_j + \text{block}_k + e_{ijk}$; where y_{ijk} is the dependent trait, α is the overall mean, treatment_i is the fixed treatment effect i , measurement_j is the fixed measurement effect (1st or 2nd), block_k is the random effect, and e_{ijk} is the residual error. Blood was collected by bleeding the animal after a blunt blow and single birds were the experimental unit for statistical analysis.

†1st, first measurement initiated immediately after blood collection; 2nd, second measurement initiated immediately after the first measurement; a measurement took ~5 minutes.