« Research Note »

Dietary Amino Acid Balance and the Precision-Fed Rooster Assay for True Amino Acid Availability

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In order to improve the utilization of feed protein for the chicken, it is important to investigate the availability of amino acids in feedstuffs. A number of ways to determine the availability have been reported^{1,2)}. A precision-fed rooster assay is one of the most useful methods³⁻⁵⁾. In the assay^{6,7)}, true amino acid availability (TAAA) is calculated using the following equation.

$$\% \text{ TAAA} = \frac{\text{Amino acid intake} - (\text{Amino acid excretion} - \text{Amino acid}_{M+E})}{\text{Amino acid intake}}$$

Where amino acid_{M+E} is metabolic and endogenous amino acid excretion by the unfed bird. Usually, a protein-free diet is used for the estimation of a metabolic fecal plus endogenous urinary amino acids. In the previous paper⁴⁾, the amino acid excretion by the unfed bird was found to be nearly equal to that by the bird fed a protein-free diet. But it is criticized to use amino acid excretion by the unfed bird for the correction to drive TAAA^{8,9)}.

The purpose of the work reported herein was to study the effect of the dietary amino acid balance on the free amino acid excretion in excreta to investigate whether or not excreta from a fasted bird can serve as an adequate measure of endogenous amino acid losses from a fed bird.

Table 1. Amino acid composition of

	DM*	CP**	ARG	HIS	ILE	LEU	LYS	MET
Yellow corn	86.6	8.7	0.45	0.25	0.32	1.00	0.28	0.17
Rough rice	86.6	6.6	0.56	0.14	0.22	0.48	0.23	0.14
Soybean meal	88.4	45.0	3.54	1.24	2.22	3.66	3.02	0.62
Groundnut meal	89.8	38.7	4.61	0.62	1.09	2.18	1.04	0.37
Fish meal	92.0	63.8	3.88	1.81	2.51	4.59	4.57	1.45
Corn gluten meal	89.3	66.9	2.19	1.27	2.62	11.03	1.17	1.87
Defatted rice bran	88.1	18.7	1.31	0.46	0.61	1.22	0.83	0.36
Dehydrated alfalfa meal	88.7	15.1	0.69	0.27	0.66	1.12	0.70	0.08

* Dry matter

** Crude protein

Materials and methods

Excreta, which were collected for the determination of amino acid availability by the precision-fed assay¹⁰⁾, were used. The assay was conducted by the TME procedures of SIBBALD⁷⁾ except length of fast prior force-feeding (30 h), length of collection period of excreta (48 h) and inputs of test materials (25 g). Each fed bird was used as its own control to correct for the amino acid losses.

The amino acid compositions of test materials are presented in Table 1.

Excreta for extraction with 0.02 N HCl were pooled from 5 replicates per treatment in the assay. 0.5 g of excreta was extracted three times with total 50 ml of 0.02 N HCl and the soluble fraction was separated by centrifugation for 20 min at 10,000 g. For free amino acid, 5 ml of the soluble fraction was concentrated to 2 ml. The solution was treated with 2 ml of sulphosalicylic acid solution (15 g/dl) to precipitate protein. The suspension was centrifuged for 20 min at 12,000 g and sample of the supernatant fraction was used for amino acid analysis. For total amino acid content, 10 ml of the soluble fraction in 0.02 N HCl was added the same volume of concentrated hydrochloric acid and was hydrolysed for 24 h at 110°C, then filtrated, concentrated under reduced pressure and redissolved in 0.02 N HCl. The amino acid content of the hydrolysate was determined with a Hitachi Amino Acid Analyser.

Results and discussion

Table 2 shows the free and total amino acid compositions of the 0.02 N HCl soluble fraction of excreta in the assay for TAAA. There were remarkable differences in the free and total amino acid contents among excreta from the birds that have given the test materials. In comparison with free amino acid content in excreta by the unfed bird, those in excreta from corn, rough rice, corn gluten meal, defatted rice bran and dehydrated alfalfa meal were nearly the same, but those in excreta from fish meal and oil-seed meals were much higher. For individual amino acids, the higher concentration of arginine was found in

feed ingredients (% as fed basis)

PHE	THR	TYR	VAL	ALA	ASP	GLU	GLY	PRO	SER
0.45	0.32	0.32	0.43	0.67	0.53	1.64	0.37	0.77	0.44
0.30	0.22	0.23	0.33	0.31	0.56	1.07	0.30	0.23	0.33
2.39	1.87	1.60	2.27	2.08	5.27	8.64	2.00	2.41	2.56
1.54	1.00	1.19	1.31	1.40	3.96	6.46	2.00	1.15	1.75
2.44	2.64	2.03	3.04	3.23	5.40	7.62	4.27	2.43	2.56
4.47	2.36	3.68	2.95	6.12	4.08	14.80	1.83	5.79	3.56
0.79	0.72	0.53	0.92	1.17	1.65	2.29	0.96	0.84	0.80
0.76	0.65	0.44	0.81	0.68	1.67	1.41	0.75	0.82	0.67

Table 2. The free and total amino acid composition of the fraction in 0.02 N HCl of excreta (mg/excreta voided for 48 h/bird)

Amino acid	Unfed	Yellow Corn	Rough rice	Soybean meal	Ground-nut meal	Fish meal	Corn gluten meal	Defatted rice bran	Dehydrated alfalfa meal
ARG free	4.3	1.5	0.8	59.6	103.6	14.4	1.6	2.5	3.2
total	7.3	5.4	5.9	68.8	135.7	46.3	12.5	15.4	13.6
LYS free	9.9	5.5	2.0	25.4	16.1	41.8	2.3	1.7	8.8
total	21.4	18.0	11.8	49.6	50.4	81.0	19.4	23.0	35.3
HIS free	4.8	2.5	1.7	6.8	6.3	18.6	—*	—	3.6
total	8.9	6.0	6.9	13.6	21.6	35.3	13.3	10.9	9.4
ILE free	1.7	1.5	1.3	2.4	3.1	5.4	2.3	2.1	4.8
total	5.7	4.6	7.2	12.2	18.4	20.5	13.7	11.5	15.8
LEU free	0.8	0.5	1.0	1.8	2.5	9.8	2.2	1.7	2.0
total	5.7	4.5	7.2	12.1	18.6	30.1	13.7	12.6	15.0
PHE free	3.1	4.7	4.5	8.3	8.5	18.1	6.3	7.1	8.6
total	6.4	6.7	7.8	14.8	15.6	25.0	12.3	11.5	17.6
THR free	3.0	1.6	1.8	5.2	4.6	12.8	3.5	4.0	6.0
total	9.2	7.4	10.7	17.2	24.3	31.5	23.2	10.7	20.2
TYR free	1.4	0.9	1.4	2.2	2.8	7.0	2.6	2.5	2.0
total	5.7	5.5	7.7	10.9	19.3	19.0	12.7	11.1	13.0
VAL free	1.5	1.4	1.6	3.5	3.7	9.5	3.2	3.8	8.2
total	4.5	3.5	5.4	10.3	19.3	25.2	10.0	10.9	14.0
ALA free	1.3	1.2	1.9	3.5	4.2	11.5	2.6	3.5	3.4
total	5.6	5.9	8.0	14.9	20.3	35.4	15.8	16.9	17.2
ASP free	2.9	5.3	2.4	10.9	8.2	15.2	10.1	11.3	13.6
total	12.9	11.3	15.6	40.9	51.6	66.2	33.1	32.4	40.7
GLU free	7.8	8.6	8.4	27.8	13.1	53.7	17.0	15.8	18.3
total	28.8	22.5	32.6	61.7	73.2	104.8	59.0	52.9	54.4
GLY free	2.0	1.2	1.8	3.3	4.2	16.3	4.0	3.5	4.0
total	15.5	14.8	27.4	29.9	60.4	68.7	41.3	42.3	39.9
PRO free	1.5	1.0	1.6	2.6	4.6	7.0	4.2	4.2	4.2
total	10.7	11.1	12.0	19.6	24.4	36.6	31.3	25.5	27.7
SER free	3.5	1.8	2.5	4.4	12.4	5.6	6.2	2.3	3.8
total	8.2	6.4	9.8	15.1	24.4	31.2	17.7	15.6	18.7
Total free	50	38	36	167	199	262	67	69	94
total	157	135	178	394	584	661	332	315	357
ORN free	3.0	3.0	2.0	26.0	22.0	26.0	3.0	1.0	4.0
Mean of (%) TAAA**		91.6	84.3	92.0	87.0	90.7	96.1	70.2	70.4

** See reference 10, * not determined

excreta from groundnut meal and soybean meal, higher concentration of lysine was also found in excreta from fish meal and soybean meal. When the birds were fed 25 g of groundnut meal (contained 250 mg of lysine and 1107 mg of arginine), 104 mg of arginine and 16 mg of lysine were excreted in excreta in the free form and 14 mg of arginine and 42 mg of lysine were excreted when fed 25 g of fish meal (1135 mg lysine, 970 mg arginine). From the finding that higher concentration of ornithine was also found in excreta which have higher

Fig. 1. Comparison of amino acid compositions of the 0.02 N HCl soluble protein fraction of excreta.

●—● Excreta by unfed birds ○—○ Corn excreta
 ▲—▲ Soybean meal excreta □—□ Alfalfa meal excreta

concentration of lysine and arginine, the excretion of the amino acids may be explained by the amino acid interaction reported by JONES *et al*⁽¹¹⁾, who showed an increase in breakdown of arginine to ornithine and increased urinary loss of arginine when fed diet with the inadequate ratio of lysine to arginine.

On the other hand, in comparison with the total amino acid content of the soluble fraction in 0.02 N HCl of excreta by the unfed bird, those in excreta from the bird fed feed-stuffs except for corn, rough rice were higher. As shown in Fig. 1, the amino acid pattern in protein (total amino acid minus free amino acid) of the fraction in excreta by the fed bird is closely similar to that in excreta by the unfed bird, consistently containing high levels of glutamic acid, aspartic acid, glycine, proline, threonine and lysine in the amino acid composition.

Amino acids in excreta by the unfed bird are considered to be originated mainly from endogenous secretions (enzymes and mucoproteins), mucosal cell debris and microbial cells. The greater part of the free amino acids is considered to be excreted in urine. SALTER and FULFORD⁽¹²⁾ suggested that the enzymes and mucoproteins are present mainly in the 0.05 M H₂SO₄ soluble fraction and the mucosal cell debris and microbial cells in the insoluble fraction of excreta.

SIBBALD⁽¹³⁾ reported that intakes of amino acids in excess of the requirement have not effect on the TAAA because the surpluses are voided as uric acid not as amino acid and PARSONS *et al*⁽¹⁴⁾ also reported that dietary amino acid balance might not be affected the results of the assay. However, it is clear from the present experiment that amino acid such

as arginine, lysine is excreted in the free form depending on the amino acid composition of dietary protein and the amino acid excretion may affect the results of the assay. Also, there were remarkable differences in the total amino acid contents among excreta from test materials. The amino acid composition of the fraction appeared to be affected by the endogenous amino acids from the enzymes and mucoproteins and the residues of dietary proteins and this results suggest a possibility that endogenous amino acid losses are affected by properties of feedstuffs as several researchers^{8,9)} reported that dietary fiber and dry matter affected excretion of endogenous amino acids by poultry.

The results of the present study indicate that the amino acid balance of dietary protein and the properties of feedstuffs affect excretion of endogenous amino acids and that the need for further researches in this area.

Acknowledgement

The author wishes to express appreciation to Mrs K. Ishikawa for her help in the work and to Nihon Nosan Kogyo Co. Ltd. for supplying feedstuffs.

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