Short communication

Apparent and true amino acid digestibility of artemia meal in broiler chicks

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Abstract

In order to determine the amino acid digestibility of artemia meal, five-week old male broiler chicks were given a semi-purified diet in which artemia meal was the sole source of protein. Apparent amino acid digestibility values of the assay diet, using ileal and excreta contents, were calculated using chromic oxide as indigestible marker. True digestibility values were calculated using endogenous output determined by feeding a nitrogen-free diet. The results showed that in determination of apparent amino acid digestibility of excreta, serine had the lowest (0.80) and methionine the highest (0.92) digestibility, while glycine had the lowest (0.88) and arginine and leucine the highest (0.95) apparent ileal digestibility. In measuring true excreta and ileal amino acid digestibility, alanine and glycine had the lowest (0.90 and 0.93) and methionine the highest (0.96 and 0.99) digestibility, respectively. In general, the site of measurement had no effect on apparent or true amino acid digestibility of artemia meal.

Keywords: Artemia meal, amino acid digestibility, excreta, ileum, broiler

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Artemia or brine shrimp is a crustacean that lives in salty habitats in about 500 zones around the world (Van Stappen, 1996). All stages of artemia from cysts and newly hatched nauplii to adult artemia are used as food sources in aquaculture, but there is little research about the use of artemia in poultry nutrition. Ras *et al.* (2002) and Zarei *et al*. (2006) used artemia meal (biomass) as a replacement for fish meal in broiler chick diets. These researchers confirmed that artemia meal could be used as a feed ingredient in poultry nutrition.

The amino acid content of artemia in different zones has been determined (Seidel *et al*., 1980; Ahmadi *et al*., 1990; Aragao *et al*., 2004) and this has shown that artemia protein is rich in several amino acids. However, it is recognized that in the diet formulation for chickens the digestibility of amino acids is required rather than the gross amino acid content of the dietary ingredients. The purpose of this study was to determine the digestibility of the amino acids in artemia meal when using the amino acid content of ileal digesta *vs*. that of excreta in the calculations.

The experiment was approved by Islamic Azad University Committee of Animal Ethics and complied with Iranian guidelines for animal welfare. A total of 100 day-old male broiler chicks (Ross 308 strain) were obtained from a local hatchery and raised in battery brooders. The birds received commercial broiler starter and grower diets from days l to 30. On day 30, forty birds of uniform body weight (1.11 $\pm$ 0.15 kg) were allocated to eight groups of five birds each, and assigned to eight cages. The study consisted of two dietary treatments, a diet containing artemia meal and a nitrogen-free diet. Therefore, each treatment was replicated four times.

The test diet was formulated to contain artemia meal as the sole source of dietary protein (Table1). Cellulose (Merck, Darmstadt, Germany) was added as a source of fibre. A nitrogen-free diet was formulated to allow for the determination of the endogenous flow of amino acids. Chromic oxide was included in all diets as an indigestible marker.

On day 30 the birds were given the diets *ad libitum* for four days and were then fasted for 24 h. The birds were then allowed to consume their diets for a one hour period (Kadim & Moughan, 1997). Excreta

Table 1 Composition of experimental feeds (g/kg air-dry basis)

Ingredient	Artemia	N-free
Artemia meal	452	-
Maize starch	379	689
Maize oil	30.0	55.0
Sucrose	80.0	170
Cellulose	20.7	45.0
Salt	-	4.0
Dicalcium phosphate	20.0	20.0
Oyster shell	10.0	10.0
Vitamin Premix	2.5	2.5
Mineral Premix	2.5	2.5
Chromic oxide	3.0	3.0

were collected for 13 h on a tray placed underneath each cage, transferred to a plastic container and frozen (-20 °C). The birds were offered the same diet *ad libitum* for a further two days. They were again fasted for 24 h and then allowed to consume their diets for one hour. Four hours after the start of the meal (Kadim & Moughan, 1997) the birds were killed by CO_2. The body cavity was opened, the ileum removed and digesta collected from the ileum. Ileal digesta of birds within a cage were pooled to provide adequate material for chemical analysis. The digesta were frozen immediately after collection at –20 °C. The excreta and digesta samples were subsequently freeze dried, finely ground and stored at –20 °C pending chemical analysis.

The amino acid concentration of the diets, the ileal digesta and excreta samples were determined using the cation-exchange HPLC system utilizing post-column OPA (orthophthaldehyde) derivitisation after 24 h hydrolysis with 6 M hydrochloric acid at 110 °C. Tryptophan was determined using alkaline hydrolysis by barium hydroxide according to the procedure of Fontaine *et al.* (1998). Dry matter (DM) (934.01) and crude protein (CP (N×6.25)) (976.05) content were determined according to AOAC (1990) procedures and chromic oxide (Cr_2O_3) determination was done using atomic absorption spectrophotometry, following the method of Fenton & Fenton (1979).

Apparent and true amino acid digestibility were calculated using the following equations (Kadim *et al*., 2002):

AA output = AA concentration in digesta or excreta x (Diet Cr_2O_3 concentration / Cr_2O_3 concentration in digesta or excreta);

Apparent AA digestibility (AID) = (AA concentration in feed - AA output (in ileum or excreta)) / AA concentration in feed;

True amino acid digestibility = (AID + Endogenous amino acid output) / Amino acid concentration in feed.

A paired t-test was used to compare ileal and excreta digestibility values. The Minitab version 13 programme was used for statistical analysis.

The crude protein and amino acid content of artemia meal are presented in Table 2. The apparent and true amino acid digestibility for the test ingredients are shown in Table 3.

When calculating apparent amino acid digestibility using excreta, serine had the lowest (0.80) and methionine the highest (0.92) digestibility, whereas glycine had the lowest (0.88) and arginine and leucine the highest (0.95) apparent digestibility when based on ileal samples. In measuring true excreta and ileal amino acid digestibility, alanine and glycine had the lowest (0.90 and 0.93) and methionine the highest (0.96 and 0.99) digestibility, respectively. Differences between excreta and ileal amino acid digestibility were not significant ($P > 0.05$).

Table 2 Nutrient composition of artemia meal (g/kg DM basis)

	Nutrients
Dry matter	924
Crude protein	541
Crude fibre	58.0
Ether extract	71.0
Ash	255
Calcium	21.0
Phosphorus	3.6
Sodium	30.0
Methionine	8.8
Cysteine	4.9
Lysine	29.4
Threonine	19.9
Tryptophan	5.7
Arginine	26.1
Isoleucine	22.7
Leucine	38.8
Valine	25.8
Histidine	10.8
Phenylalanine	24.9
Glycine	22.3
Serine	18.9
Alanine	31.0
Aspartic acid	42.2
Glutamic acid	58.8

Although the amino acid digestibility values of artemia meal based on ileal collections were higher than values calculated from excreta collections, differences were not significant. This suggests that microbial activity in the hindgut of broilers did not affect the amino acid digestibility. A likely explanation is that because of the high digestibility of amino acids in artemia meal, there was little amino acid reaching the hindgut to be affected by the microflora (Ravindran *et al*., 1999). Wallis & Balnave (1983) reported that in high quality proteins, digestion and absorption of amino acids were completed in the upper parts of the small intestine, leaving little available for microorganism fermentation. Kadim *et al.* (2002) showed that, when comparing excreta and ileal digestibility in highly digestible ingredients, there were no significant differences between the two methods of determination.

Among indispensable amino acids, threonine had the lowest apparent digestibility. The same result has been reported with other animal ingredients (Raharjo & Farrell, 1984; Ravindran *et al*., 1999; Kadim *et al*., 2002; Huang *et al*., 2006) and is likely the result of high concentrations of threonine in endogenous protein. Endogenous secretions consist of digestive enzymes and mucin that are rich in threonine, serine, aspartic acid and glutamic acid (Salter & Fulford, 1974; Ravindran *et al*., 2004) and can affect the apparent threonine digestibility. As shown in this study, using true amino acid digestibility can eliminate the underestimation of threonine digestibility.

Parsons (1984) found that hindgut fermentable carbohydrates caused an increase in amino acid secretion in intact rather than in caecectomised birds. As suggested by Sauer & Ozimek (1986), the amount of energy-yielding carbohydrates reaching the hindgut appears to determine whether net degradation and net synthesis of amino acids will take place. The lower digestibilities based on excreta in the present study suggested that some fermentable carbohydrates reached the large intestines, though that the quantities were too low to cause significant differences between calculations based on excreta *vs.* ileal collections. On the other hand, the higher ileal value could be due also to contamination of excreta with scurf and feathers.

Table 3 Apparent and true amino acid digestibility (coefficients) of artemia meal determined by sampling either excreta or ileal contents

Amino acid	Apparent digestibility				True digestibility			
	Excreta	Ileal	s.e.m.[1]	P [2]	Excreta	Ileal	s.e.m.	P
Methionine	0.92	0.94	0.004	NS	0.96	0.99	0.004	0.09
Lysine	0.88	0.92	0.007	NS	0.92	0.96	0.007	NS
Threonine	0.85	0.90	0.013	NS	0.93	0.98	0.011	NS
Tryptophan	0.88	0.94	0.014	NS	0.90	0.97	0.017	NS
Arginine	0.89	0.95	0.008	0.09	0.93	0.98	0.008	0.08
Isoleucine	0.88	0.94	0.011	NS	0.92	0.98	0.011	NS
Leucine	0.89	0.95	0.009	0.06	0.94	0.98	0.009	NS
Valine	0.87	0.93	0.010	NS	0.93	0.98	0.010	NS
Histidine	0.89	0.93	0.007	NS	0.95	0.97	0.007	NS
Phenylalanine	0.87	0.94	0.009	0.09	0.92	0.97	0.009	NS
Glycine	0.81	0.88	0.015	NS	-	0.93	-	-
Serine	0.80	0.89	0.018	NS	0.91	0.97	0.017	NS
Alanine	0.85	0.91	0.014	NS	0.90	0.94	0.014	NS
Aspartic acid	0.86	0.91	0.010	NS	0.91	0.94	0.005	0.09
Glutamic acid	0.87	0.93	0.014	NS	0.93	0.95	0.013	NS
Total	0.85	0.92	0.010	0.09	0.94	0.96	0.011	NS
CP (N × 6.25)[3]	0.81	0.89	0.013	NS	0.89	0.94	0.012	NS

NS - non significant; [1] Standard error of the means; [2] Probability.
CP – crude protein; N - nitrogen. [3] The values (protein digestibility) were not corrected for uric acid.

In this study, crude protein digestibility was lower than total amino acid digestibility. It is probably due to the high content of chitin in artemia meal. Chitin is the crustacean exoskeleton polysaccharide and consists of N-acetyl glucosamine residues that forms part of the protein complex, and is considered to be poorly digested in the gastrointestinal tract of chickens (Austin *et al*., 1981; Khempaka *et al*., 2006). As a result it would be voided in excreta, thereby decreasing crude protein digestibility. It seems that N-acetyl glucosamine had little effect on the amino acid digestibility in artemia meal. The protein digestibility values in the present study are in agreement with values reported by Zarei *et al*. (2005).

Conclusion

Results of the present study showed that site of measurement do not have a significant effect on the apparent or true amino acid digestibility of artemia meal. It demonstrated that the amino acids in artemia meal have relatively high digestibilities, suggesting that artemia meal can be considered as a protein source in poultry nutrition.

Acknowledgements

We offer our thanks to the chairman, vice president and personnel of the Iranian Animal Science Research Institute, Karaj Islamic Azad University, for making this research possible.

References

Ahmadi, M.R., Leibovitz, H. & Simpson, K.L., 1990. Nutrition composition of brine shrimp (*Artemia urmiana*). Comp. Biochem. Physiol. 95, 225-228.

AOAC, 1990. Official Methods of Analysis (15th ed.). Association of Official Analytical Chemists, Inc., Washington, D.C., USA.

Aragao, C., Concicao, L.E.C., Danis, M.T. & Fyhn, H.J., 2004. Amino acid pools of rotifers and artemia under different conditions: nutritional implication for fish larvae. Aquaculture 234, 429-445.

Austin, P.R., Brine, C.J., Castee, J.E. & Zikaksi, J.P., 1981. Chitin: New facets of research. Science 212, 749-753.

Fenton, T.W. & Fenton, M., 1979. An improvement procedure for determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59, 631-634.

Fontaine, J., Bech-Anderson, S., Butikofer, U. & Froidmont-Gortz, I., 1998. Determination of tryptophan in feed by HPLC – development of an optimal hydrolysis and extraction procedure by the EU Commission DG XII in three international collaborative studies. Agrobiol. Res. 51, 97-108.

Huang, K.H., Ravindran, V. & Bryden, W.L., 2006. Comparison of apparent ileal amino acid digestibility of feed ingredients measured with broilers, layers and roosters. Poult. Sci. 85, 625-634.

Kadim, I.T. & Moughan, P.J., 1997. Development of an ileal amino acid digestibility assay for the growing chicken - Effects of time after feeding and site of sampling. Br. Poult. Sci. 38, 89-95.

Kadim, I.T., Moughan, P.J. & Ravindran, V., 2002. Ileal amino acid digestibility assay for growing meat chicken - Comparison of ileal and excreta amino acid digestibility in chicken. Br. Poult. Sci. 44, 588-597.

Khempaka, S., Mochizuki, M., Koh, K. & Karasawa, Y., 2006. Effect of chitin in shrimp meal on growth performance and digestibility in growing broilers. J. Poult. Sci. 43, 339-343.

Minitab, 2000. Minitab User's Guide, Version 13. Minitab Inc., State College, P.A., USA.

Parsons, C.M., 1984. Influence of caecectomy and source of dietary fibre or starch on excretion of endogenous amino acids by laying hens. Br. J. Nutr. 51, 541-548.

Raharjo, Y. & Farrell, J.D., 1984. A new biological method for determining amino acid digestibility in poultry feedstuffs using a simple cannula and the influence of dietary fiber on endogenous amino acid output. Anim. Feed Sci. Technol. 12, 29-45.

Ras, M.B.B., Agh, N., Yahyazadeh, M.Y., Sahebkalam, J. & Hojjati, M., 2002. Chemical composition and nutritive values of Artemia urmiana in broiler ration. In: Proc. World Aquaculture Soc., April 23-27, Beijing, China.

Ravindran, V., Hew, L.I., Ravindran, G. & Bryden, W.L., 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. Br. Poult. Sci. 40, 266-274.

Ravindran, V., Hew, L.E., Ravindran, G. & Bryden, W.L., 2004. Endogenous amino acid flow in avian ileum: quantification using three techniques. Br. J. Nutr. 92, 217-223.

Salter, D.N. & Fulford, R.J., 1974. The influence of gut microflora on the digestion of dietary and endogenous protein: Studies of the amino acid composition of excreta of germ free and conventional chicks. Br. J. Nutr. 32, 625-637.

Sauer, D.N. & Ozimek, L., 1986. Digestibility of amino acids in swine: results and their practical applications: a review. Livest. Prod. Sci. 15, 367-388.

Seidel, C.R., 1980. Amino acid composition and electrophoretic protein patterns of artemia from five geographical locations. In: The Brine Shrimp Artemia. Eds Persoone, G., Sorgeloos, P., Roels, O. & Japers, E., Universa Press, Wetteren, Belgium. pp. 375-382.

Van Stappen, G., 1996. Introduction, biology and ecology of Artemia. In: Manual on the Production and Use of Live Food for Aquaculture. Eds Lavens, P. & Sorgeloos, P., Food and Agriculture Organization of the United Nations, Rome, Italy. pp. 80-88.

Wallis, I.R. & Balnave, D., 1984. A comparison of amino acid digestibility bioassays for broilers. Br. Poult. Sci. 25, 389-399.

Zarei, A., Mirhadi, A., Shivazad, M. & Lotfollahian, H., 2005. Comparative study of protein digestibility of artemia meal and fish meal under condition of in vitro and in vivo. Pajouhesh & Sazandegi, 68, 10-18. (in Farsi, English abstract).

Zarei, A., Shivazad, M. & Mirhadi, A., 2006. Use of Artemia meal as a protein supplement in broiler diet. Inter. J. Poult. Sci. 5 (2), 142-148.