Portal Absorption of Feed Oligo-peptides in Chickens

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ABSTRACT : The effect of duodenal infusion with feed oligo-peptide solution on portal absorption of amino acids was investigated in poultry under unanaesthetized conditions. Four peptide solutions were used in the experiment: enzymatic hydrolysates from fish meal, soybean meal, cottonseed meal and rapeseed meal proteins with average molecular weights less than 3,000 Da and 1,000 Da, respectively. Intestinal absorptions of these oligo-peptide solutions were compared by determining the concentration of free amino acid (FAA) in portal blood after the duodenal administrations of oligo-peptide solutions. Absorptive intensity and balance were used to estimate the intestinal absorption rate of amino acids. The absorptive intensities of amino acids were highest for the fish and soybean meal oligo-peptides. The ratios of amino acids absorbed in the portal blood from fish and soybean meal oligo-peptides were more similar to the composition of the infused amino acids than that observed from the cottonseed and rapeseed meal oligo-peptides. A positive correlation was found between absorption rate and proportion of PAA in the oligo-peptides. The higher absorption rate could be contributed to the higher proportion of peptide bound amino acids (PAA). The results suggest that fish and soybean meal protein are significantly more easily hydrolyzed into oligo-peptides ($p<0.05$) in the gastrointestinal tracts of poultry and as such can be utilized more effectively by body tissues. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1277-1280)***

Key Words : Chickens, Oligo-peptide, Intestinal Absorption, Duodenal Infusion, Portal Amino Acid

INTRODUCTION

It has been recognized in recent years that the end products of feed protein found in animal digestive tracts were mostly oligo-peptides. These play a dominant role in the absorption of amino acids (Kim, 1972; Matthews et al., 1975b). Evidence exists suggesting that these peptides are transported by systems independent of those responsible for transporting free amino acids. Peptide transports are active due to higher absorption speed, low levels of energy consumption, and less competition for carriers than those of free amino acids making peptides, especially dipeptides and tripeptides, effective donors of amino acids (Burston et al., 1972; Matthews et al., 1975b; Webb, 1990). They can be rapidly transported into intestinal mucosal cells and hydrolyzed before being absorbed as their constituent amino acids into blood flow (Webb et al., 1990; Daniel et al., 1994).

There have been many studies on the absorption of peptides, most of which indicated higher absorptive speed and intention than FAAs (Matthews et al., 1972; Hara et al., 1984; Silk et al., 1985; Rerat et al., 1988; Le et al., 1997). The peptides used in these experiments, however, were mostly standard dipeptides, tripeptides or enzymatic hydrolysates of some special proteins such as casein, albumin and lactoglobulin. These peptides have special properties and therefore have some limitation. *In vivo* or *in vitro* experiments Feng et al. (2002) and Savoie et al. (1989) reported that the proportions of PAA hydrolyzed from quality feed proteins are higher than those from poor feed proteins. This suggests that the amount of PAA in protein hydrolysate might be a reason behind the difference of feed amino acid availability. The present investigation used duodenal infusion to compare the absorption of oligo-peptides hydrolyzed from common feed proteins in order to better understand the factors that affect the digestible utilization of feed proteins of different qualities.

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MATERIALS AND METHODS

Preparation of infused oligo-peptide solutions

The oligo-peptides utilized in this study were enzymatic hydrolysates of four common feed proteins (fish, soybean, cottonseed and rapeseed meal proteins). They were *in vitro* hydrolyzed using pepsin and trypsin with the following hydrolysis procedure. Eight grams of feed sample was placed in a 800 ml flask with stopper. To this was added 80 mg pepsin (Sigma Comp. USA, activity 1:10,000) dissolved in 80 ml pH 2.0 HCl. The flask was placed horizontally in an oscillator (120 r/min) and incubated at 37°C for 6h. A few drops of 50% NaOH were added to adjust pH to 7.0. Then 200 mg trypsin (BD Comp. USA, activity 1:250) was added with 400 ml pH 7.6 phosphate buffer. The incubation was continued for an additional 18 h. 120 ml 5% (w/v) trichloroacetic acid (TCA) was then added and the hydrolysate centrifuged at 8,000 g, 4°C for 30 min. The supernatant was ultra filtered to discard the fractions with average molecular weights <3,000 Da and <1,000 Da, one ml of the hydrolysate fraction was analyzed for FAA and total amino acid (TAA) concentration (before and after 6 N HCl hydrolysis at 110°C for 24 h) by high performance liquid chromatography (HPLC) following derivation by FMOC.

Table 1. Molar percent of each amino acids and PAA proportion in oligo-peptide solutions

AA	F3	S3	C3	R3	F1	S1	C1	R1
Asp	6.58	6.08	4.76	4.27	4.46	5.34	5.35	4.54
Ser	2.74	6.35	3.11	6.99	3.77	8.74	4.14	5.94
Glu	5.70	8.90	6.19	9.79	8.72	8.01	8.37	10.48
Thr	2.06	2.48	1.81	2.03	2.19	4.06	2.05	2.24
Ala	6.52	9.22	4.16	5.43	5.10	8.25	3.97	4.77
Gly	12.21	16.77	18.25	17.84	18.66	20.23	19.19	6.76
Tyr	2.46	2.16	2.64	4.60	0.69	0.44	3.64	5.06
Pro	9.47	6.82	6.35	4.00	3.84	7.79	4.75	18.41
Val	19.90	8.02	26.19	24.85	32.00	6.03	15.20	23.67
Phe	1.78	5.51	8.08	3.40	0.91	5.35	9.36	4.10
Ile	1.19	3.61	2.56	2.07	0.52	2.66	3.11	0.83
leu	3.47	4.69	2.82	2.53	5.16	5.24	4.15	2.22
His	19.48	17.02	11.20	10.41	10.91	14.96	14.23	9.12
Lys	6.43	2.37	1.87	1.78	3.09	2.89	2.49	1.87
Sum	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
PAA/TAA (%)	87.85	86.05	83.96	75.74	77.16	73.95	71.15	70.34

F3, S3, C3 and R3 represent hydrolysate fraction of <3,000 Da of fish meal, soybean meal, cottonseed meal and rapeseed meal, respectively.
F1, S1, C1 and R1 represent hydrolysate fraction of <1,000 Da of the same ingredients.

Depending on the concentration of TAA and FAA, appropriate volumes of eight oligo-peptide fractions from four feed protein hydrolysates were measured and evaporated to dryness at 65°C, then dissolved in 5 ml of distilled water to ensure equal TAA amounts (100 umol). Eight such oligo-peptide solutions were prepared for infusion of per chicken. The molar ratio of each amino acid and PAA proportions in the eight oligo-peptide solutions are shown in Table 1.

Experimental procedure

The chickens were deprived of food for 24 h prior to infusion. A glucose vitamin added to water (50 g per chicken) was provided *ad libitum* during the deprivation period. The experiment was performed under unanesthetized and unrestrained conditions. A small opening was made at the right abdomen near keel follicle. The duodenum was removed and 5 ml of oligo-peptide solution was administered from the upper site of duodenum using a syringe. The duodenum was then replaced in the abdomen. The birds were allowed to move about normally. Different aminals were sampled at each period of time.

Sampling of plasma

Abdominal wall was opened rapidly and 10 ml of portal blood collected and placed in a centrifuge tube with a pre-added anticoagulant of heparin sodium. The whole blood was centrifuged at 5,000 g, 4°C for 20 minutes. Plasma was added to bring the volume to 0.6 mol/L Perchloric acid (PCA) followed by another centrifuge at 8,000 g, 4°C for 15 minutes. The supernatant solutions of two chickens in one replicate were mixed at equal proportion and the final plasma sample stored at -20°C prior to analysis.

Experiment 1 : Fifty four 6 wk-old chickens of average weight (1.5±0.1 kg) were randomly divided into 9 groups with 3 replicates per group and 2 chickens for each replicate. One group was infused with 5 ml of 0.9% NaCl solution as a control. The other eight groups were all administrated 5 ml S3. Portal blood was collected at 0, 5, 10, 15, 20, 30, 40, 60 and 90 minutes, and the plasma FAA concentration was determined.

Experiment 2 : Forty two 6 wk-old chickens of average weight (1.5±0.1 kg) were randomly divided into 7 groups with 3 replicates per group and 2 chickens for each replicate. Seven groups were duodenal infused with 5ml of F3, C3, R3, F1, S1, C1 and R1, respectively. Portal blood was collected at 20 minute after infusion according to results obtained in experiment 1, and the plasma FAA concentration was determined. Absorptive intensity and balance were then calculated and analyzed in comparison with the proportion of PAA present.

Analysis of plasma FAA

A 5 ml plasma sample was evaporated to dryness at 65°C and 2 ml inner standard of bezoar acid added prior to derivation by 9-Fluorenylmethyl Chloroformate before HPLC determination of amino acids.

Met, Trp, Arg, and Cys were not measured. As such, the FAA in this study refers to 14 amino acids and TAA refers to the sum of the 14 amino acids.

Estimate of absorptive efficiency of amino acids

Two indexes of absorptive intensity and balance were used to estimate the portal absorptive efficiency of amino acids. The intensity of intestinal absorption for each amino acid was calculated from its elevated concentration in portal blood in the 20 minutes before and after infusion and expressed as slope. The intestinal absorptive balance of the amino acids absorbed was expressed as X^2, according to the following equation:

Table 2. Changes in plasma total FAA concentration after infusion of S3 (n=3)

min	0	5	10	15	20	30	40	60	90	SEM	P Value
FAA concentration (umol/L)	1,759.47	2,098.14a	2,596.01b	3,301.36c	4,632.85d	3,334.00c	2,466.54e	1,749.44	1,966.32k	9.59	<0.01

Means within a line lacking different lower letters differ ($p<0.05$).

Table 3. Comparison of absorptive intensity and balance of AA from different peptides (n=3)

Portal FAA (umol/L)	Control	F3	S3	C3	R3	F1	S1	C1	R1	SEM	P Value
Asp	117.56a	352.38 b	465.29c	224.50d	139.86	284.59e	133.57	264.27f	125.42k	2.70	<0.05
Ser	146.29a	353.18 b	404.68c	304.83	307.73	326.90d	306.51	344.21e	312.07	2.08	<0.05
Glu	45.31d	124.84 a	150.60c	149.09c	125.02a	171.29	127.81ab	124.99a	132.88b	1.47	<0.05
Thr	124.28	250.44ab	370.09c	250.13ab	252.51a	312.14d	178.42e	245.01b	202.24f	1.04	<0.05
Ala	145.94	286.99a	419.57b	228.09c	266.72d	215.34e	186.93f	176.37k	245.97m	2.23	<0.05
Gly	216.92	565.42a	583.02b	462.56c	390.30d	515.02e	278.66f	311.44k	245.92m	2.94	<0.05
Tyr	49.17b	265.75e	185.28d	200.90	195.25	100.39a	101.76a	119.68e	140.72f	2.02	<0.05
Pro	133.99	256.52a	359.32d	170.38c	164.71c	138.99	233.71e	261.03a	191.12f	2.05	<0.05
Val	222.56a	470.62c	407.78	448.90c	405.21	505.17d	543.19e	277.09f	300.15k	2.60	<0.05
Phe	43.11	210.02f	105.65a	132.33c	122.15b	101.47a	115.06ab	136.33cd	144.70de	1.65	<0.05
Ile	60.75	128.79a	205.00e	115.49b	104.27bc	117.65ab	100.81cd	151.32f	104.80bd	1.58	<0.05
leu	41.31a	180.02b	209.89c	157.73	139.55d	77.89e	154.54	167.01f	124.96k	1.79	<0.05
His	253.33a	842.20b	468.09c	519.10d	680.55e	369.27	630.95f	372.31	457.69k	3.34	<0.05
Lys	158.95	388.51m	318.59a	286.30abc	250.96bdef	293.85adg	301.39aek	252.48cfgk	231.07f	2.07	<0.05
∑FAA	1,759.47f	4,655.67a	4,632.85a	3,650.33 b	3,544.80bc	3,529.95bd	3,393.31be	3,203.53def	2,959.70f	7.33	<0.05
∑Slope		144.81	143.67	94.54a	89.27ab	81.69c	88.52b	72.20d	60.01e	1.43	<0.05
X^2		42.20	41.88	64.15a	63.26a	69.34c	88.19b	86.47b	92.87d	1.15	<0.05

Means within a line lacking different lower letters differ ($p<0.05$).

$$X^2=\Sigma(fi-Fi)^2/Fi, \quad (1)$$

where fi is the ratio of intensity of each amino acids indicated as percent value. Fi is the molar ratio of each amino acids administrated.

$$\text{slope (umol/L·min)}=(C_1-C_0)/t.$$

where C_1 and C_0 indicated total FAA concentration in portal plasma in a period of time after and before infusion of oligo-peptide.

A lower X^2 value indicates that the balance of amino acids was better maintained in the absorptive ratio of each amino acid from the administrated oligo-peptide.

Statistical analysis

Statistical differences among the oligo-peptide infusion groups were determined using Duncan's multiple range test (Duncan, 1955), and the correlation analyzed by linear regression. All statistical analyses were performed using the SPSS 10.0 program.

RESULTS

Change of portal plasma FAA after duodenal infusion of S3 solution

Table 2 indicates the change of total FAA concentrations in portal blood after the infusion of S3. FAA concentrations immediately increased and reached a maximum 20 min after administration ($p<0.05$), then decreased to the control value after 60 to 90 min.

The effect of duodenal infusion of different oligo-peptide solutions on the intestinal absorptive efficiency of amino acids

Table 3 shows the FAA concentrations in experiment 2. Compared with the control, the portal plasma FAA concentrations increased significantly after infusion of eight oligo-peptide solutions (data of S3 are from Experiment 1). The F3 and S3 groups were significantly higher than other groups, with C3 higher than C1 and R1 ($p<0.05$). There was no difference between C3 and R3, F1 or S1, and R1 and C1.

Table 3 also indicates that the absorptive efficiencies were significantly different among the eight groups. The absorptive intensities of amino acids from F3 and S3 are markedly higher than the other groups ($p<0.05$).

Regression analysis of PAA proportion in the administrated oligo-peptides and intestinal absorptive efficiency of amino acids

Two linear regression processes were carried out on the PAA/TAA using the oligo-peptide solution as the independent variable and absorptive intensity and balance as dependent variables. The coefficients between the proportion of PAA in the oligo-peptide solution and absorptive intensity and balance are 0.92 and 0.93.

Regression equations were Y=4.23X-234.09 and Y= -2.70X+280.24.

DISCUSSION

The intestinal absorption rates of peptides and corresponding amino acid mixtures were compared in many experiments. Silk et al. (1985) used a method of intestinal perfusion with a double balloon catheter. Hara et al. (1984) installed cannulation of the portal vein and placed the tip of the catheter behind the neck of rat skin. Studies of Matthews et al. (1972), Guowei Le et al. (1997) used the similar method of duodenal infusion as the present investigation except that theirs were performed under anesthesia disturbing the movement of intestines or blood flow. This experiment aimed to a rapid and sensitive determination and to minimize the individual differences in animals and consequently to allow a good comparison among infusion groups of different oligo-peptide solutions.

The peptides used in reported experiments were mostly standard dipeptides, tripeptides, or enzymatic hydrolysates of some special proteins such as casein, albumin and lactoglobulin. While the oligo-peptide solutions used in this study were prepared from enzymatic hydrolysis of fish meal, soybean meal, cottonseed meal and rapeseed meal to simulate the gastrointestinal conditions of poultry. Accordingly, the results may better illuminate the intestinal absorption of digestive products of common feed proteins.

To estimate the intestinal absorption rate of amino acids, an index of absorptive intensity and balance was used according to that of Hara et al. (1984). Both slope and X^2 were calculated from FAA concentrations taken 20 minutes after infusion. This may have had a slight effect on the recycled amino acids, although the groups were still comparable. In order to discuss the factors affecting the rate of absorption, correlations were assessed between PAA content in the oligo-peptide solution and absorptive intensity and balance. The rate of intestinal absorption was positively correlated with the proportion of PAA in the oligo-peptide solution. Thus, the higher rate of absorption of amino acids from F3 and S3 oligo-peptide solutions could have contributed to their higher PAA proportions.

The ratio of absorptive intensity of each amino acid from those oligo-peptide solutions containing more PAA was in better balance. The loss of absorptive balance among amino acids in groups having less PAA may be due to the competition from amino acids on the brush border membrane. Based on these results, the proteins from fish meal and soybean meal were easily hydrolyzed to oligo-peptides by enzymes in animal gastrointestinal tracts. These oligo-peptides provided more rapidly absorbed sources of amino acids from intestine and maintained a better balance after absorption compared to hydrolysates from cottonseed meal and rapeseed meal protein. This may be the reason why fish meal and soybean meal protein supply better availability of amino acids and are more efficiently utilized by body tissues in monogastinal animals than cottonseed meal and rapeseed meal protein.

REFERENCES

Burston, D. J., M. Addison and D. M. Matthews. 1972. Uptake of depeptide containing basic and acidic amino acids by rat small intestine *in vitro*. Clin. Sci. 43:823.

Daniel, H., M. Boll and U. Wenzel. 1994. Physiological importance and characteristics of peptide transport in intestinal epithelial cells. In: (Ed. W. B. Souffrant and H. Hagemeister). VIth international Symposium on Digestive Physiology in Pigs.Vol. 1:1-7. Dummerstorf Pub.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1-42.

Feng, X.Y., C. Ji and H. Jiang. 2002. Relationship between amino acid availability and hydrolyzed peptide quantity in poultry feed. Journal of China Agricultural University 7(1):107-113.

Hara, H., R. Funabili, M. Iwata and K. I. Yamazaki. 1984. Portal absorption of small peptides in rats under unrestrained conditions. J. Nutr. 114:1122.

Kim, Y. S., W. Birtwhistle and Y. W. Kim. 1972. Peptide hydrolases in the brush border and soluble fractions of small intestinal mucosa of rat and man. J. Clin. Invest. 51:1419.

Le, G. W., Y. H. Shi, J. Xie, Z. H. Wang and F. Yang. 1997. The effect of duodenal infusion of casein hydrolysate- COP or equivalent AA mixture on the intestinal amino acids absorption and portal plasma peptides of chicks. Acta Zoonutrimenta Sinica. 9(4):14-23.

Matthews, D. M. 1975. Intestinal absorption of peptides. Physiol. Rev. 55:527.

Matthews, D. M. Rate of peptide uptake by small intestine. 1972. In: (Ed. K. Ellion and M. O'Connor) CIBA Symposium: peptide transport in bacteria and mammalian gut. pp. 71-88. Associated scientific publishers, Amsterdam.

Rerat, A., C. S. Nunes, F. Mendy and L. Roger. 1988. Amino acid absorption and production of pancreatic hormones in anaesthetized pigs after duodenal infusions of a milk enzymatic hydrolysate or of free amino acids. Br. J. Nutr. 60:121-136.

Savoie, L., R. Charbonneau and C. Parent. 1989. *In vitro* amino acid digestibility of food proteins as measured by the digestion cell technique. Plant-foods for human nutr. 39:93-107.

Silk, D. B. A., G. K. Grimble and R. G. Rees. 1985. Protein digestion and amino acid and peptide absorption. Proc. Nutr. Sci. 44:63.

Webb, K. E., Jr. J. C. Matthews and D. B. DiRienzo. 1992. Peptide absorption -a review of current concepts and future perspectives. J. Anim. Sci. 70:3248.

Webb, K. E. 1990. Intestinal absorption of protein hydrolysis products: A review. J. Anim. Sci. 68:3011-3022.