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Comparison of Ileal Digested Production of Parental Rice and Rice Genetically Modified With Cowpeas Trypsin Inhibitor

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Objective To compare the ileal digestibility of protein and amino acids in parental rice and rice genetically modified with *sck* gene. **Methods** Six experimental swines were surgically fixed with a simple T-cannula at the terminal ileum and fed with parental rice and rice genetically modified with *sck* gene alternately. The ileum digesta were collected and analyzed for determination of apparent and true digestibility of protein and amino acids. **Results** The apparent and true digestibility of protein was similar in these two types of rice. Except for the apparent digestibility of lysine, there was no difference in the apparent and true digestibility of the other 17 amino acids. **Conclusion** The digestibility of protein and amino acids is not changed by the insertion of foreign gene, so it can meet the request of "substantial equivalence" in digestibility of protein and amino acids.

Key words: Genetically modified rice; Apparent digestibility; True digestibility

INTRODUCTION

With the rapid development of genetically modified foods in the world, the safety assessment of these novel foods is becoming the first task for governments, scientists, and consumers. At the same time, nutritional assessment is an important aspect of the safety assessment of genetically modified foods. Because of the insertion of foreign genes, it should be concerned whether the content of nutrients, anti-nutrients and the bioavailability of important nutrients in genetically modified foods are influenced[1].

Rice is the main food for the Chinese people, but the great amount of pests in field can eat more than ten million tons of rice a year and makes a great losts for agriculture. So the genetic modification of rice for resisting pests is of great importance.

The popular gene used in most countries for anti-pests is BT gene, which was obtained from *Bacillus thuringiensis*, but it can resist only a few types of pests. Cowpea trypsin inhibitor from cowpea seeds, a member of the serine protease inhibitor family, has a wide range of insect resistance and high safety[2]. So *cpti* gene is widely used as the insect-resistant gene in plant genetic engineering. But in recent years, the use of this gene has been restricted because of the low accumulation of the foreign *cpti* protein in transgenic plants[3]. After years of hard work, researchers from Institute of Genetics and Developmental Biology, the Chinese Academy of Sciences have made some modifications to increase the stability of *cpti* protein in plants by fusing a signal sequence at the 5′ end and an endoplasm reticulum retention signal gene at the 3' end of *cpti* gene[4]. The new gene is called *sck* gene. ELISA test indicates that the accumulation level of foreign *cpti* protein in *sck* transgenic plants are 2-4 times higher than that in *cpti* transgenic plants. At the same time, *sck* transgenic plants have higher resistance to pests[4].

Recently, this *sck* gene has been transferred into a parental rice cultivar (hybridized Minghui 86 paddy of China) for anti-pest. The results in farm show that this genetically modified rice (GM rice) can resist most pests in field. Many researches have been done on the safety assessment of this GM rice, and the initial results showed that no adverse or teratogenic effects are observed[5-6]. Chemical analysis and feeding study on experimental mini-pigs have also been done in our laboratory[7] to compare the nutritional composition and feeding value of this GM rice to parental rice. In this study, in order to find the bio-availability of protein in this GM rice, the ileal digestibility of protein and amino acids in GM rice was measured and compared with that of the parental rice.

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MATERIALS AND METHODS

Materials

The experimental paddy used in this study was grown in the experimental field of the Chinese Academy of Sciences in Fujian Province. The experimental field is located at east longitude 116′40 degree and north latitude 26′06 degree. The parental paddy (Minghui 86 paddy) and GM paddy (Minghui-86 paddy genetically modified with *sck* gene) were harvested between October 23-27, 2002. These two types of paddy were stored at a dry and ventilated depot. Before study, all paddies were hulled by the same procedure.

Animals

Ten crossbred (Duroc×Landrace) emasculative adult male swines were obtained from the Experimental Animal Base of the Agriculture University of China, Beijing. Animals were housed individually in adjustable metabolism cages. After 7 days of adaptation, all the swines were surgically fixed with a simple T-cannula at the terminal ileum following the procedures described by Qi-Guang HUO [8]. After surgery, the swines were immediately returned to the metabolism cages and allowed a 14-day recovery period. During this period, animals were fed twice daily with a corn and soybean meal-based grower diet and had free access to water. Six swines growing well were chosen as experimental animals for the following digestion study.

Diets

All the rice was ground thoroughly through a 0.9 mm mesh screen, then 0.1% di-chromium trioxide was added in the diet as an indicator[9]. The composition of different diets is shown in Table 1.

Experimental Procedure

There were three periods for this experiment[10], 7 days each period, 5 days for adaptation to the dietary treatments, and 2 days for collecting ileum digesta. In the first period, all swines were fed with 5% casein diet and ileum digesta were collected for determining the endogenous amino acid flow. In the second period, 6 swines were divided into 2 groups, 4 pigs were fed with GM rice diet and 2 pigs with parental rice diet, and the procedure was the same as the first one. In the third period, the diet for the animals was changed, 4 pigs were fed with parental rice and 2 pigs were fed with GM rice diet. Ileal digesta were collected from 8:00 to 18:00. A foam rubber bag was fixed to the cannula with a rubber band for digesta collecting. The bag was removed every 2 hours and immediately frozen at -20℃ until further analysis.

TABLE 1

Composition of Experimental Diets

Ingredients	Parental Rice Diet	GM Rice Diet	5% Casein Diet
Parental Rice	97.9	-	-
GM Rice	-	97.9	-
Casein	-	-	5.0
Sucrose	-	-	20.0
Cellulose	-	-	5.0
Corn Starch	-	-	65.85
Di-calcium Phosphate	-	-	1.7
Sodium Chloride	-	-	0.35
Soybean Oil	1	1	1
Vitamins and Elements Premix*	1	1	1
Di-chromium Trioxide	0.1%	0.1%	0.1%
Total	100	100	100

Note. *Vitamin and element premix can provide the following amount of nutrients for 1 kg diet: vitamin A, 11000 IU; vitamin D_3, 1503 IU; vitamin E, 44.1IU; vitamin K, 4.0 mg; riboflavin, 5.2 mg; pantothenic acid, 20.0 mg; niacin, 26 mg; vitamin B_{12}, 0.01 mg; Mn, 35.0 mg; Fe, 100 mg; Zn, 90.0 mg; Cu, 16.5 mg; I, 0.30 mg; Se, 0.30 mg.

Sample Preparation and Analyses

After experiment, digesta for individual pigs in each period were stirred and freeze-dried, then ground through a 0.28 mm mesh screen and thoroughly mixed before analysis. Nitrogen content in rice, diet and digesta was determined by Kjeldahl method[11]. Chromium was determined by atomic absorption spectrometry after acid digestion[12]. Tryptophan was hydrolyzed by 4.2 mol NaOH to analyze amino acids and determined by fluorospectro-photometric method. Cysteine was oxidized by performic acid and the other 16 amino acids were hydrolyzed by 6 mol HCl and analyzed with an automatic amino acid analyzer[13].

Calculations[14] and Statistical Analysis

The apparent ileal digestibility values of N and amino acids were calculated according to the following equation:

Apparent digestibility(AD) $= 100-[(AA_{digesta}\times Cr_{diet})/(AA_{diet}\times Cr_{digesta})]\times 100$

Where AA_{diet} and $AA_{digesta}$ are the concentrations (mg/kg) of nitrogen and amino acids in the diet and digesta, and Cr_{diet} and $Cr_{digesta}$ are the concentrations (mg/kg) of chromium oxide in the diet and digesta respectively.

The endogenous amino acid flow is calculated by the following equation:

Endogenous amino acid flow (EF) = $AA_{digesta} \times (Cr_{diet} / Cr_{digesta})$

Where $AA_{digesta}$ is the concentration of nitrogen and amino acids in the digesta and Cr_{diet} and $Cr_{digesta}$ are the concentrations (mg/kg) of chromic oxide in the diet and digesta when all the animals are fed with 5% casein diet.

The true ileal digestibility value of N or amino acids was calculated according to the following equation:

True digestibility (TD) = $AD+(EF/AA_{diet}) \times 100$

All data were analyzed with SPSS 11 software. The apparent and true digestibility values of N and amino acids in the GM rice diet were compared to those in parental rice diet by *t*-test. There was a significant difference in the two types of diet when $P<0.05$.

RESULTS

Protein, Amino Acids and Phytate Content in Parental and GM Rice

The appearance and odor of parental and GM paddy were similar, and after being hulled, the weight of a thousand grains of rice was 22.43 g for parental rice and 22.46 g for GM rice. The protein, amino acids and phytate content in parental and GM rice were analyzed and compared in this study, and the results showed that the contents of these nutrients were similar and comparable in these two types of rice (Table 2).

Apparent Ileal Digestibility Values of GM and Parental Rice

All the swines remained healthy and grew normally throughout the study. Before experiment, their initial weight was 62.17±5.04 kg. The mean intake of GM and parental rice diet was 2.12±0.18 kg/day and 2.17±0.23 kg/day, respectively.

The apparent ileal digestibility of protein and amino acids in GM and parental rice is shown in Table 2. The apparent digestibility of crude protein in parental rice was similar (70.10%±6.73%) to that in GM rice (69.05%±3.94%). The apparent digestibility rates of most amino acids were 60%-80% and the values were similar between GM rice and parental rice ($P>0.05$). The apparent digestibility of lysine in parental rice was significantly higher than that in GM rice ($P=0.003$, Table 3).

TABLE 2

Comparison of Protein, Amino Acids, Phytate Content in GM, and Parental Rice

Item	Parental Rice (g/100 g)	GM Rice (g/100 g)
Crude Protein	7.5	7.4
Arginine	0.44	0.42
Histidine	0.12	0.13
Isoleucine	0.27	0.27
Leucine	0.57	0.60
Lysine	0.21	0.21
Methionine	0.24	0.22
Phenylalanine	0.45	0.46
Threonine	0.25	0.25
Tryptophan	0.30	0.28
Valine	0.54	0.52
Alanine	0.37	0.39
Asparagine	0.60	0.59
Cysteine	0.23	0.25
Glutamic acid	1.27	1.31
Glycine	0.31	0.31
Proline	0.35	0.35
Serine	0.34	0.33
Tyrosine	0.30	0.28
Phytate	0.278	0.309

TABLE 3

Apparent Digestibility of GM and Parental Rice (%) (n=6)

Item	Parental Rice	GM Rice	*P* Value
Crude protein	70.10±6.73	69.05±3.94	0.749
Arginine	84.06±3.23	83.03±4.12	0.639
Histidine	79.73±4.26	77.24±3.59	0.298
Isoleucine	60.84±9.64	52.87±7.84	0.147
Leucine	73.14±6.18	71.83±4.43	0.682
Lysine	75.59±4.67	66.09±3.52	0.003*
Methionine	79.67±7.66	83.19±4.99	0.368
Phenylalanine	80.13±4.70	81.64±3.50	0.541
Threonine	59.57±10.00	54.46±5.86	0.306
Tryptophan	74.76±11.31	74.76±10.80	1.000
Valine	77.77±6.44	79.84±2.99	0.490
Alanine	69.31±8.77	70.50±4.47	0.777
Asparagine	72.95±6.00	69.35±4.07	0.252
Cysteine	75.44±9.67	80.64±4.34	0.296
Glutamic Acid	80.72±5.80	80.17±3.64	0.848
Glycine	57.93±10.13	55.39±10.04	0.672
Proline	73.35±6.60	66.90±9.15	0.192
Serine	71.42±6.86	68.41±4.72	0.396
Tyrosine	73.55±7.41	72.95±4.07	0.865

Note. *The digestibility of the two types of rice was significantly different ($P<0.05$).

Ileal Digestibility Values of GM and Parental Rice

The endogenous amino acid flows at the terminal ileum were determined by feeding 5% casein to experimental swines and the results are showed in Table 4.

So the true digestibility of protein and amino acids in GM and parental rice was calculated and the results are shown in Table 5.

TABLE 4

Endogenous Amino Acid Flows (g/kg Intake)

Item	EF	Item	EF	Item	EF
Arginine	0.42	Phenylalanine	0.38	Cysteine	0.49
Histidine	0.35	Threonine	0.14	Glutamic acid	1.75
Isoleucine	0.56	Tryptophan	0.76	Glycine	1.13
Leucine	0.69	Valine	0.66	Proline	1.68
Lysine	0.54	Alanine	0.66	Serine	0.96
Methionine	0.18	Asparagine	1.03	Tyrosine	0.25
N	2.82				

Note. EF means endogenous flow.

TABLE 5

True Digestibility of GM And Parental Rice(%)(*n*=6)

Item	Parental Rice	GM Rice	*P* Value
Crude protein	91.59±9.63	94.46±4.02	0.523
Arginine	91.76±4.33	92.74±3.84	0.687
Histidine	100.00*	100.00#	0.529
Isoleucine	77.09±11.66	73.36±7.93	0.532
Leucine	83.07±7.33	83.53±4.46	0.898
Lysine	93.63±9.40	91.06±3.51	0.551
Methionine	87.17±7.66	91.15±4.84	0.307
Phenylalanine	87.38±5.40	89.49±3.08	0.426
Threonine	83.78±13.13	84.86±5.86	0.859
Tryptophan	90.31±9.78	97.67±4.76	0.129
Valine	89.17±6.99	92.30±2.93	0.347
Alanine	83.44±10.45	87.58±4.49	0.400
Asparagine	86.31±8.58	86.44±3.90	0.939
Cysteine	98.09±8.83	100.00*	0.270
Glutamic Acid	92.02±7.44	93.60±3.68	0.651
Glycine	88.01±13.75	91.84±10.04	0.594
Proline	100.00*	100.00*	0.925
Serine	94.74±9.85	97.80±4.56	0.512
Tyrosine	81.23±7.83	82.10±4.15	0.813

Note. *If the data were >100%, it was shown as 100%.

The results in Table 5 showed that the true digestibility of crude protein in parental and GM rice was more than 90% and there was no significant difference in these two types of rice. Except for isoleucine, the true digestibility for the other 17 amino acids was more than 80%, and there were no significant differences between the two types of rice.

DISCUSSION

The digestibility of protein and amino acids in food reflects their bioavailability in body, which can be expressed by the apparent digestibility (AD) and true digestibility (TD), differing in endogenous flow of N or amino acids. Since TD measurements takes into account the metabolic nitrogen or amino acids without dietary origin, it is more accurate than AD value. AD values increase with the increasing of protein intake, whereas TD values are independent of protein intake[15].

Methods for determining the digestibility values of amino acids are various, such as total tract measurement (balance method), ileal cannula methods, *etc*. In recent years the determination of protein and amino acid bioavailability in rats by the balance method (total tract measurement) has been criticized by many scholars. Because of the possible microbial modifications of undigested and unabsorbed nitrogenous residues in the large intestine, the pattern of nitrogen excretion can be modified by the microflora in the large intestine. This modification may cause over-estimation of the digestibility of protein and amino acids[15]. Therefore, measuring the disappearance of amino acids from the small intestine (ileal recovery) may provide more accurate data on their bioavailability[16].

For determining the endogenous flow of protein and amino acids, animals can be fed with a protein-free diet or a diet with just enough highly digestible protein. Protein-free diet method is the traditional method, but was criticized by some scholars in recent years because it underestimates the endogenous flow of amino acids. Many experts have recommended highly digested protein diet (such as 5% casein diet) to prevent excessive loss of body protein[10]. So in this study, 5% casein diet was used to determine the endogenous amino acid flow in animals.

The results in this digestion study showed that the apparent and true digestibility of protein and most amino acids in GM rice was similar to that in parental rice. The true digestibility of protein in GM rice and parental rice was 91.59±9.63 and 94.46±4.02 respectively, being a little higher than the data published in 1985[18]. This difference may be attributed to the different metabolized method or different subjects. The apparent digestibility of amino acids was 60%-80%, and the true digestibility was 80%-100%, the data were similar in most amino acids. The apparent digestibility of lysine in GM rice was significantly lower than that in parental rice (P=0.003), but the true digestibility of this amino acid was similar in the two types of rice. Whether the difference is correlated with the insertion of foreign genes should be further studied.

From the results of this digestion experiment, we can conclude that the digestibility of protein and amino acids in GM rice is not changed by insect of the foreign *sck* gene. But the final conclusion of "substantial equivalence" of this GM rice to parental rice should be supported by further experiments.

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