

Research Article

Decomposition of Proteolytic Enzymes of Earthworm to Cottonseed Meal

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Abstract: The objective of this research was to determine: 1) the decomposition of proteolytic enzymes of earthworm to cottonseed meal; 2) the amino acid content in earthworm, cottonseed meal and the complex which mixed proteolytic enzymes with cottonseed meal. The results showed that the enzymatic activity of proteolytic enzymes in earthworm to cottonseed meal was high. After mixed with proteolytic enzymes, the amino acid content in cottonseed meal increased. Various proportions of essential amino acids is more reasonable. Therefore, using proteolytic enzyme can effectively improve the feed quality of cottonseed meal.

Keywords: Amino acid, cottonseed meal, earthworm, proteolytic enzymes

INTRODUCTION

Protein content of cottonseed is up to 37~45%, it belongs to soy protein of high quality and its nutritional value is much higher than the cereal proteins (Lawson, 1998). The essential amino acids (except methionine) of cottonseed meal have reached the UN Food and Agriculture (FAO) recommendations (Agarwal, 1996). Crude protein content in cottonseed meal is 2.89% higher than soybean meal, calcium and phosphorus content were also 0.05 and 0.19% higher than soy (Gao *et al.*, 2013). And it is rich in all kinds of amino acids, essential amino acids and non-essential amino acids can both be comparable with soybean, so it is high-protein feed resources. But because cottonseed protein structure is very close and has large molecular weight, after ingestion by animals, the low molecular weight nitrogen-containing products are not easy to be digested or absorbed. And coupled with cottonseed meal containing gossypol and other anti-nutritional factors, it limits the cottonseed protein is widely used in the food industry.

Earthworm is an important ecological component of many soils (Bouwman 2007). The protein content in earthworm is high, accounted for fresh weight of 8 to 10%, accounting for dry weight of 56 to 66% (Khalil *et al.*, 1996). The active ingredients in earthworm show a strong hydrolysis activity on protein. Hydrolytic enzymes in earthworm can break down protein into earthworm hydrolyzate, which is of a high nutritional value.

The objective of this study is to obtain the decomposition of proteolytic enzymes of earthworm to

cottonseed meal and the amino acid content in earthworm, cottonseed meal and the complex which mixed proteolytic enzymes with cottonseed meal.

MATERIALS AND METHODS

Earthworms: In this experiment, earthworms were *Eisenia fetida*. They brought to the laboratory within one week in a plastic container. Earthworms were maintained in plastic boxes containing sterilized loam. Cow dung was provided as feed daily and earthworms were free to feed ad libitum. Healthy earthworms of about 60 days old, with a well-developed clitellum, were used for exposure experiments. They were starved for one day to allow depuration of their gutcontents before being used in experiments.

Preparation of earthworm hydrolyzate: Fresh earthworms 100 g, rinse with tap water clean, drain earthworm surface water and put it into conical flask, stamped. Hydrolysis under 50°C about 5 h until the color changed into brown. The earthworm hydrolyzate of earthworm preserved in refrigerator, which the temperature was 4°C.

Preparation of cottonseed protein: Mix 300 g pulverized cottonseed meal with 1800 mL distilled water under 50°C. Add 0.1mol/L NaOH until the pH is 10-12. Centrifugal 10 min under 1500 r/min, the supernatant was collected. Adjusted the pH to 5-6 and standing 30 min. Then centrifugal 15 min under 3000 r/min, the dry precipitate was collected.

Table 1: Diameter of transparent ring under different response time

Response time (h)	1	2	3	4	5
Diameter (mm)	8.2	9.7	10.8	12.3	14.3
Response time (h)	6	7	8	9	10
Diameter (mm)	16.5	17.1	16.8	16.2	16.3

Preparation of agarose protein flat: The pH of PBS buffer value was 8.9, cottonseed protein 0.3 g was dissolved in 15 mL PBS buffer. Heat PBS buffer (pH = 7.2), put 0.425 g agarose in PBS buffer, heating until the agarose dissolved completely. Adding 15 mL cottonseed protein solution into agarose solution, put the mixture in dishes, rushed to the edge of the bubble, solidified at room temperature.

Punch 4 holes in each dish; the diameter of the hole is 3 mm. Drop the earthworm hydrolysis into the hole. Keep the dishes in 50°C with different the reacting time.

RESULTS

The results of agarose protein plate method: Around agarose protein plate holes, the transparent rings can be seen clearly. Specific data are shown in Table 1. Coomassie Brilliant Blue 250 can bind specific by van der Waals forces with proteins, but can not bind with

amino acid. Proteolytic enzyme in earthworm's hydrolyzate can break down cottonseed proteins around the holes, when protein is broken down into amino acids; coomassie blue is eluted under the effect of glacial acetic acid, while other portions without being decomposed into amino acids could not be decolorized. Therefore, the transparent circles that can be seen clearly around the holes describe Earthworm proteolytic enzymes can break down Cottonseed Protein, the optimum time of decomposition reaction must be 7 h.

The results of hydrolysis: After 7 h reacting, cottonseed protein is broken down by earthworm proteolytic enzymes and produce free amino acids, the degree of hydrolysis is of high value, it describes that proteolytic enzymes in Earthworms hydrolysis has a high enzymatic activity on cottonseed protein.

Amino acid content in reactant: After reaction, amino acid content in reactants increased from 26.51 to 44.22%. The components of amino acid were shown in Table 2.

Among them, except the contents of Phenylalanine, Valine, Serine, Cystine, Tyrosine and Arginine in reactants decreased, the contents of other amino acids increased in different degrees (Table 3).

Table 2: Crude protein and amino acid content (g/100 g sample)

Components	Amino acid in earthworm	Amino acid in cottonseed meal	Amino acid in reactant
Crude protein	64.33	40.63	49.58
Leucine	5.21	2.18	3.31
Isoleucine	2.91	1.31	1.79
Phenylalanine	2.33	1.99	1.09
Alanine	4.23	1.67	2.64
Aspartate	6.89	/	4.84
Valine	2.94	3.01	2.64
Threonine	3.32	1.23	1.41
Serine	3.28	1.65	1.46
Glutamate	11.24	7.94	12.82
Glycine	3.48	1.57	2.42
Lysine	4.04	1.36	2.27
Cystine	0.68	0.73	0.65
Tyrosine	2.41	1.03	0.84
Histidine	1.74	1.03	1.25
Methionine	1.15	0.63	0.83
Arginine	4.26	3.49	2.59
Proline	2.59	1.17	1.37
Total	62.70	26.51	44.22

Table 3: The ratio of amino acid content as a pig feed

Components	The ratio to Lysine in pig feed	The ratio to Lysine in cottonseed protein	The ratio to Lysine in reactants
Lysine	1.00	1.00	1.00
Leucine	0.95~1.00	1.60	1.45
Isoleucine	0.54~0.56	0.96	0.79
Threonine	0.64~0.68	0.90	0.62
Arginine	0.36~0.40	2.56	1.14
Valine	0.67~0.69	2.21	1.16
Histidine	0.31~0.32	0.76	0.55
Cystine+Methionine	0.56~0.59	1.00	0.65
Phenylalanine+Tyrosine	0.92~0.94	2.22	0.85

CONCLUSION

Earthworm's proteolytic enzymes showed high enzymatic activity to cottonseed protein. Using earthworms to decompose cottonseed meal, amino acids were increased significantly. Compared with cottonseed meal, the total amino acids in reactants raised 66.8% and the proportion of essential amino acids is more reasonable. Therefore, the use of earthworm's proteolytic enzyme hydrolyzed cottonseed protein can effectively improve the feed quality of cottonseed meal.

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