ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 7 (10): 984-989, 2008
ISSN 1682-8356

Efficacy of Hydrolytic Enzyme Preparation from *Aspergillus* Spp. on the Fattening Broiler Chicken Fed with Corn Soybean Based Diets

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Abstract: The influence of supplementing enzyme preparations made of three *Aspergillus* species (*A. niger* DB106, *A. oryzae* NM1 and *A. niger* XP) isolated from Vietnam on growth and performance of broiler chickens were investigated individually and in combination. In a completely randomized design, 270 of 30 day-old broiler chicks (Luong Phuong strain) were divided into 9 treatments with 3 replicate of 10 chicks per pen. Treatments were a dietary control (group 1) without enzymes and diets supplemented with enzyme preparations individually or in combinations to produce the other treatments (group 2-9). At the end of the feeding trial, 6 chicks per replicate were sacrificed to evaluate carcass and meat characteristics. The results as experiment finished of 5 weeks later demonstrated that the body weight gains were improved in approximately 6-16% with enzyme addition individually and from 14.5-27.2% ($P < 0.05$) with addition of enzyme combinations. The effect of the combination of enzyme preparations from three species of *Aspergillus* showed the highest efficacy on BWG of chickens (27.2% compared to control group), which were much higher than the effect of Green enzyme (11.6%) from China that is available in Vietnam market. Feed conversion ratio (FCR) was reduced in all experimental groups compared to the control ($P < 0.05$). The meat quality and carcass characteristics of chickens in all groups were not altered under the effect of the enzyme preparations. This finding demonstrated that these enzyme preparations are safe and very prospective preparation for the fattening broiler chickens.

Key words: Broiler chickens, *asperigillus* enzymes, body weigh gain, carcass, feed conversion ratio

Introduction

Nowadays, many farmers tend to over stimulate their animals with various substances such as hormones and toxic chemicals that affected public health. The addition of exogenous enzymes into the diet for improving the performance of animals is one of the alternative measures to solve the problem and it has been extensively studied and applied during the past decades as a means of enhancing and increasing the effectiveness of nutrient utilization (Acamovic, 2001). Enzyme supplementation has dramatically increased all over the world, but predominantly in pigs and poultry diets (Officer, 2000; Bedford, 2000; Cowieson and Adeola, 2005). The positive effect of single or multiple enzymes on growth and performance of broiler chickens have been intensively studied and reported (Zhengkang, 1997; Hew *et al*., 1998; Alam et *al*., 2003; Cowieson and Adeolae, 2005; Watee *et al*., 2006; Omojola *et al*., 2007). The use of exogenous enzymes as feed additives is still called much attention and lots of researches are undergoing.

The genus *Aspergillus* is one of the most important filamentous fungal genera. Many species of the genus are important candidates for biotechnology industries since they produce a large variety of extracellular enzymes of significant industrial importance. *A. niger* has even been granted the GRAS (Generally Regarded As Safe) status in certain industrial production processes by the Food and Drug Administration of the US government. Therefore, most of the feed enzymes commercialized on the markets are products of *Aspergillus*.

Recently, in Vietnam, commercial feed enzyme products such as phytase and mixtures of hydrolytic enzymes (xylanase, cellullase, amylase and protease...etc.) have been commonly found in the market. Most of commercial feed enzyme products were produced by foreign companies such as BASF, Kemine, Adisso, Rhode...etc, which based on the results of studies on feed ingredients and feeding conditions that are not the same as these in Vietnam. Utilization of these products in feed industry in Vietnam now is very limited (mainly by the foreign feed mills with good equipment and technology). Studies on feed enzymes for animal diets in Vietnam so far are still very little and there no report on the effect of the enzyme feed produced in Vietnam on chickens. This study investigated the effect of enzyme preparations made of *A. niger* DB 106 (Cong and Hang, 2005), *A. oryzae* NM1 (Ly and Hang, 2006) and *A. niger* XP phytase (Xuan and Hang, 2006) supplemented to corn soy-bean based diets on growth performance and feed utilization efficiency of fattening chickens.

Materials and Methods

Enzyme production was performed in Biotechnology and Microbiology laboratory, Hanoi National University of

Education (HNUE), Vietnam. Enzyme trials on animals were conducted in Center for study and trials of animal feed, National Institute of Animal husbandry, Thuy Phuong, Hanoi, Vietnam.

Animal basal diet: A corn/soybean-based basal diet was formulated to be nutritionally marginal in terms of energy, Ca, and P and provided only 2963.1-3006 kcal of ME/kg, 0-2.0% Ca and 0.75% available P with as presented in the Table 1.

Animals and experiment arrangement: The experiment was done according to completely randomized block design (CRBD) with total of 270 chicks of Luong Phuong strain aged of 2 weeks (135 males and 135 females), divided into 9 groups, which were housed in floor pens (10 animals/pens, triplicate) and were fed with proprietary grower basal diet for two weeks and then a grower diet with or without addition of the enzyme preparations at various combinations as indicated in Table 2.

Measurements and data analysis: Feed offered and refused was recorded daily for average daily feed intake (ADFI) calculation. Body weight (BW) of chickens in all groups was measured at the beginning and at the end of each feeding ages to calculate average daily gain (ADG) and total body weight gain (BWG). Standard procedures were followed. The collected data was analyzed for ANOVA using Microsoft Excel. Individual mean value was compared by Fisher's to show the difference at confident level of 95%.

Results and Discussion

Effect on growth performance: The results of growth performance of chicken were presented in Table 4.

At ages of 4-6 weeks, body weight gain of chickens in all enzyme treatment groups were increased compared to the control diet. The supplementing A. *niger* DB 106 enzyme preparation individually (group 4) and in combination with *A. oryzae* NM1 enzyme (group 7) showed high effect on growth and performance of chickens compared to the control group. However, the differences at this stage do not get supported statistical significance ($P > 0.05$).

At the ages of 8-12 weeks, the growth of chickens in all enzyme treatment groups were markedly increased compared to the control one.

Addition of individually enzyme preparation either of *A. niger* XP (Group 2) or *A. ozyrae* NM 1 (Group 3) could improve the body weight gain by 5.6% and 7.2%, respectively, compared to control one. However, they were not statistically significant ($P > 0.05$). Whereas, inclusion of *A. niger* DB 106 enzyme preparation individually (group 4) showed high efficacy on chicken body weight gain (16%) compared to control group ($P < 0.05$).

Table 1: Grower basal diet formulations (g/kg) and calculated nutrient provision

Ingredient s	Grower basal diet g/kg
Maize	642.5
Soya bean meal 44% CP	190.0
Rice bran	90.0
Fish meal 60% CP	50.0
Vitamin-mineral premix No 8	2.5
Minerals	20.0
NaCl	2.0
Lysine	1.0
Calculated provision	
ME (Kcal)	2963.1
Crude Protein %(CP)	18.07
ME/CP	163.17
Ca%	1.08
Total P %	0.75
Crude fiber %	3.7
Lyzin % 0.1	
Methionin %	0.49
Methionin + Cyst %	0.75
NaCl %	0.22

Note: Composition of Vitamin-mineral premix No 8: Vitamin A + D: 470.000 UI; Vitamin E + K: 1.08 g; Vitamin: B_1 + B_2 + B_6: 420 mg; Vitamin B_{12} + H_2 + Folic acid: 48.9 mg; Niacin: 1.2 g; D. Calpan: 400 mg; Fe + Cu: 1.86 g; Zn + Mn: 7.5 g; I+ CO + Se: 84 mg.

In the groups where the chickens fed with diets supplemented with various combinations of two enzyme preparations such as *A. niger* DB 106 with *A. niger* XP (group 5); *A. niger* XP with *A. ozyrae* NM 1 (group 6) and *A. niger* DB 106 with *A. oryzae* NM 1 (group 7) the BWG of chickens were clearly increased (from 14.9-25.7%) compared to control one ($P < 0.05$). Among them, the most effective combination is that of *A. niger* DB 106 with *A. oryzae* NM 1 which could result in BWG improvement of 25.7% higher than control diet. The combinations of *A. niger* XP preparation with either *A. niger* DB 106 or *A. oryzae* NM 1 were less effective. This finding is in good agreement with the result obtained (Group 2), which showed *A. niger* XP enzyme preparation having less influence on chicken BWG than *A. niger* DB 106 and *A. oryzae* NM 1.

Supplementing of the 3 studied *Aspergillus* enzyme in combination to the control diet (group 8) could enhance the BWG by 27.2% compared to the control group and 2.4% compared to the group 7, where the chickens were fed with combination of *A. niger* DB 106 and *A. oryzae* NM 1 preparation. In group 9, where chickens were fed with control diets supplemented with Green enzymes (China) the BWG increased 11.3% compared to group of control diet.

Feed consumption and conversion ratio: The difference of average feed intake (ADI) of chickens in all groups was not statistical significant ($P > 0.05$), which indicates the supplementing enzymes preparation did not improve feed intakes.

Table 2: Experiment arrangement

Experimental arrangements	Grower basal diet	Content of enzyme added (% ≈ U/kg feed)
Group I (Control I)	Control basal diet 1-(GBD)	0
Group II	GBD + *A. niger* XP phytase	0.05% ≈ 400 IU phytase/kg
GroupIII	GBD + *A. oryzae* NM 1 enzymes	0.1% ≈ 635 IU amylase/kg
Group IV	GBD + *A. niger* DB 106 enzyme	0.1% ≈10360 U xylanase/kg
Group V	GBD + *A. niger* DB 106 enzyme	0.1%
	+ *A. niger* XP phytase	0.1%
Group VI	GBD + *A. oryzae* NM1 enzymes	0.1%,
	+ *A. niger* XP phytase	0.05%
Groups VII	GBD + *A. niger* DB 106 enzyme	0.1%,
	+ *A. oryzae* NM 1 enzymes	0.1%
Group VIII	GBD + *A. niger* DB 106 enzyme	0.1%,
	+ *A. oryzae* NM 1 enzymes	0.1%,
	+ *A. niger* XP phytase	0.05%
Group IX (control 2)	GBD + Green Enzyme (China)	0.05% (30000 IU xylanase/kg, 30000 IU beta-glucanase/kg

Note: The composition of the enzyme preparations were given in the Table 3. All diets for chickens in 9 groups were in the meal mass form (not pellet form).

The difference of Food conversion ratio (FRC) in all experimental groups was clearly reduced. However, the differences of reducing FRC of groups 2 and 4 compared to control group were not statistical significance ($P > 0.05$).

In inclusion of *A. niger* DB 106 individually or in combination either with *A. niger* XP, or with *A. ozyrae* NM 1, or with both of them, the FRC were decreased in 11.2%,11.4%, 12.4% and 12.7% ($P < 0.05$), respectively, meanwhile FRC in the group 9, where chickens fed with diet in addition of Green enzyme from China was reduced to 11.7%.

It is concluded that the supplementing combination of three *Aspergillus* spp. enzyme preparations showed much higher efficacy on BWG and FRC compared to both control diet (negative control) and diet with addition of Green enzyme (China) as well.

Exogenous enzyme supplementation has been used commercially to improve nutrient digestibility of corn-soybean diets and the effectiveness of the addition of microbial enzymes on growth and performance of broiler chickens have been reported. Zhengkang (1997) reported the addition of enzymes to diets increased the body-weight gain of chicks by 2.3-18.9% (with average of 10.8%, $P < 0.01$) and feed conversion efficiency also improved significantly. Cowieson and Adeola (2005) found that body weight gain followed a similar trend, showing an improvement of approximately 6-7% with either enzyme individually and a 14% improvement with a combination of phytase and xylanase and protease. The data of Watee *et al.* (2006) showed the BWG in Thai Native chickens fed with the diet supplemented culture extract from *Thermoascus aurantiacus* SL 16 W increased in 16%.

In this study, the individual enzyme preparation from the *Aspergillus* spp. could improve the BWG in the range of the reported results (6-18%). However, combinations of the enzymes added to basal diet gave faster growth rate than other compared products did (Zhengkang, 1997; Cowieson and Adeola, 2005; Watee *et al.*, 2006). It might be due to the high synergistic action of the enzymes in mixtures of the preparations used. The exact mechanism of synergistic actions of enzymes in *Aspergillus* spp. preparations leading to high efficacy of promoting growth and performance of chickens could not be clearly understood since our *Aspergillus* crude enzyme preparations consisting of several hydrolytic enzymes as mentioned in the Table 3.

However it could be deduced that due to presence of high content of cellulase, xylanase and phytase in these enzyme preparations (Table 3), the fibers (lingo-cellulose and hemi-cellulose) and phytate of feed were efficiently degraded and released digestible sugars and inorganic P, respectively. Consequently, the proteins and other nutrients were released and readily exposed to the action of animal hydrolytic enzymes as well as added fungal enzymes to release the amino acids and easy absorbed nutrients. Presence of alpha-galactosidase may lead to the degradation of the melibiose (galactose-alpha-1, 6-glucose), raffinose (galactose-alpha-1, 6-sucrose) and stachyose (galactose-alpha-1, 6-raffinose) that are abundance in soybean diet. The action of alpha-galactosidase improves quality of feed as well as elicits the anti-nutrition factors either. One of the distinguished feature of these enzyme preparations is presence of high content of raw-starch-digesting amylases which could more effectively digest starch in not cooking form of the diets supplied, whereas the action of soluble starch digesting amylase are well performed when starch were cooked. Moreover, these raw-starch amylases were composed of alpha - amylase and glucoamylase, which could digest starch almost completely to glucose (data not showed), while under the action of alpha-amylase of the stomach mainly maltose and maltooligosaccharides were produced. Presence of *Aspergillus* spp. raw starch digesting glucoamylase might be accelerating the completion

Table 3: Composition of the enzyme preparations used in this study

Enzymes	*A. niger* DB106 U/g	*A. oryzae* NM1 U/g	*A. niger* XP U/g	Green Enzym EZQ-201 U/g
Endoxylanase	1,192.8	23.0	0	60.000
Beta-Xylosidase	2.4	0.5	1.0	-
Alpha-Galactosidase	1.4	1.45	0.9	-
CMC-ase	13.9	3.53	2.7	1.200
Raw starch digesting alpha-Amylase	11.0	635.5	0	-
Glucoamylase	21.3	0.2	0	
Protease	0	4.0	0	1.000
Phytase	0	0	800	-
Mananase:	ND	ND	ND	4.000
Beta-glucanase	ND	ND	ND	50.000

●The enzyme activities of *Aspergillus* spp. were determined at 40°

Table 4: Effect of the enzyme preparations on growth performance of Luong Phuong chickens

		Experiment groups					
Variables	Age (weeks)	Group 1 Mean (g)	Group 2 Mean (g)	Group 3 Mean (g)	Group 4 Mean (g)	Group 5 Mean (g)	Group 6 Mean (g)
Live weight (kg/bird)	4	432.7^{a}	430.5^{a}	426.7^{a}	431.7^{a}	430.5^{a}	428.5^{a}
	6	578.1^{a}	558.6^{a}	571.3^{a}	593.6^{a}	648.6^{a}	598.6^{a}
	8	906.1^{a}	913.6^{a}	937.8^{a}	1013.9^{b}	1033.6^{b}	985.5^{a}
	10	1275.0^{a}	1226.7^{a}	1268.3^{a}	1343.2^{b}	1346.7^{b}	1290.8^{a}
	12	1528.9^{a}	1577.5^{a}	1604.5^{a}	1702.6^{b}	1697.5^{b}	1671.2^{b}
Total body weigh gain (kg/bird)		1096.2	1147.0	1174.0	1270.9	1261.0	1239.2
Total body weighs gain (%)		100.0	105.6	107.2	116	116.1	114.9
ADFI (kg/bird/day)		71.8^{a}	72.1^{a}	72.0^{a}	73.2^{a}	70.4^{a}	73.4^{a}
ADG (g/bird/day)		19.6^{a}	20.6^{a}	20.6^{a}	22.4^{b}	21.9^{b}	22.2^{b}
FCR(kg/feed/kg gain)		3.66^{a}	3.5^{a}	3.48^{a}	3.27^{b}	3.21^{b}	3.3^{a}
FCR reduced (%)		0.0	4.6	5.1	11.2	11.4	11.1
Live rate (%)		100	100	100	100	100	100

		Experiment groups				
Variables	Age (weeks)	Group 7 Mean (g)	Group 8 Mean (g)	Group 9 Mean (g)	± SEM	P-value
Live weight (kg/bird)	4	431.7^{a}	429.3^{a}	435.8^{a}	8.0	0.83
	6	593.6^{a}	693.6^{a}	567.8^{a}	18.4	0.065
	8	1013.9^{b}	1083.9^{b}	950.3^{a}	39.6	0.024
	10	1475.8^{b}	1505.8^{b}	1320.6^{a}	60.8	0.001
	12	1809.6^{c}	1824.6^{c}	1659.4^{b}	70.2	0.005
Total body weigh gain (kg/bird)		1377.9	1395.3	1223.6		
Total body weighs gain (%)		125.7	127.3	111.6		
ADFI (kg/bird/day)		74.2^{a}	73.8^{a}	68.1^{a}	2.7	0.38
ADG (g/bird/day)		25.2^{c}	25.98^{c}	21.9^{b}	1.25	0.005
FCR(kg/feed/kg gain)		2.94^{b}	2.84^{b}	3.12^{b}	0.3	0.002
FCR reduced (%)		12.4	12.7	11.7		
Live rate (%)		100	100	100		

$^{a-c}$Means in a column without a common superscript are significantly different ($P < 0.05$).

Abbreviations: ADFI: average daily feed intake; ADG: average daily weight gain: FCR: feed conversion ratio; GBD: Grower Basal diet Experiment arrangement: 1) Control group fed with GDB; 2) GBD + *niger* XP phytase; 3) GBD + *A. oryzae* NM 1 enzymes; 4) GBD + *A. niger* DB 106 enzyme; 5) GBD + *A. niger* DB106 + *A. niger* XP phytase; 6) GBD + *A. oryzae* NM 1 enzymes + *A. niger* XP phytase; 7) GBD + *A. niger* DB 106 enzyme + + *A. oryzae* NM 1 enzymes; 8) GBD + *A. niger* DB 106 enzyme + + *A. oryzae* NM 1 enzymes + *A. niger* XP phytase GBD and 9) GBD + Green Enzyme (China).

of starch digestion process in the digestive system, consequently, improving the growth rate.

Such actions were well documented in literature. In most cases, the exogenous enzymes are known to improve feed intake and utilization of high fiber diets and diets rich In non-starch polysaccharide, which, apart from being resistant to the digestive enzymes, also reduce the digestibility of other dietary components like proteins and hence reduce the performance of the animal. Bedford (1997) reported that exogenous enzyme supplementation resulted in an overall improvement in nutrient digestion and a reduction in endogenous amino acid losses.

Hew *et al.* (1998) demonstrated that exogenous xylanase significantly improved ileal nitrogen and amino acid digestibility, and apparent metabolizable energy

Table 5: The influence of enzyme preparations on meat quality and carcass characteristics of Broiler Chickens

	Experiment groups (n = 10 birds/groups)										
Parameters	CG	I	II	III	IV	V	VI	VII	VIII	SEM	P- value
Body meet/BW (%)	74.7	74.4	74.1	74.7	74.3	74.6	75.7	74.1	75.2	1.1	1.00
Breast muscle/BW (%)	19.5	19.8	18.5	20.5	19.3	19.5	20.6	20.5	21.3	0.38	0.93
Thigh muscle /BW (%)	20.5	21.6	20.2	20.5	19.8	19.5	20.8	21.0	22.8	0.44	1.00
Thigh + Breast /BW (%)	40.0	40.4	39.7	41.0	38.0	39.0	41.4	40.0	42.1	0.32	0.74
Abdominal fat /BW (%)	1.7	1.7	2.0	1.8	2.1	2.0	1.9	1.8	1.8	0.04	0.78
Liver/BW (%)	2.39	2.25	2.39	2.22	2.1	2.30	2.22	2.10	2.5	0.08	0.52
Hear/BW (%)	0.73	0.72	0.73	0.69	0.7	0.73	0.69	0.71	0.66	0.02	1.00
Pancreas/BW(%)	0.32	0.30	0.32	0.33	0.31	0.32	0.33	0.30	0.28	0.01	1.00
Spleen/BW (%)	0.27	0.26	0.27	0.25	0.22	0.27	0.25	0.22	0.25	0.02	1.00
Proventriculus/Live (%)	0.44	0.47	0.44	0.46	0.44	0.45	0.50	0.42	0.50	0.002	0.86
Gizzard/Live weight (%)	2.17	2.0	2.17	2.03	2.17	2.17	2.03	2.1	2.06	0.12	1.00
Caecae/Live weight (%)	0.64	0.64	0.64	0.62	0.61	0.64	0.62	0.60	0.60	0.01	1.00

(AME) in wheat for broiler chickens. Bhat (2000) demonstrated that the use of hydrolases eliminates ANF present in grains, degrades certain cereal components in order to improve the nutritional value of feed and to supplement animals' own digestive enzymes. Many reports suggest that the xylanase can be improving growth performance due to xylanase functioning in the hydrolysis of arabinoxylan and starch. Addition of microbial phytase to diet minimizes the need for supplementation with inorganic phosphorus and excretion of phosphorus in manure. Mondal *et al.*, 2007, reported that the body weight and the body weight gain of the broilers fed soybean meal control and low phosphorus plus phytase diet were heavier and the feed conversion ratio of broiler fed on low phosphorus plus phytase 500 PU/kg was significantly better. The percentage of tibia ash, Ca and phosphorus and retention of Ca and P was significantly increased by the addition of microbial phytase to low phosphorus diet. The interaction of phytase and hydrolase enzyme is well documented. It showed that there is positive effect of interactions of those enzymes and no negative interactions of those enzymes were found, indicating that both types of enzymes may be used together in feeds based on corn, wheat, or barley (Juanpere *et al.*, 2005). Those data are indicating the digestion of such components in animal digestive systems was considerably improved.

Effect of enzyme supplementation on carcass characteristics of broiler chickens: All the animals used in the experiments were alive over the experiment period of 12 weeks. It indicates that the enzymes supplemented in diet had no negative effect on chicken survival. This finding is in agreement with similar results reported (Cowieson and Adeola, 2005; Watee *et al.*, 2006; Omojola and Adesehinwa, 2007).

Six chickens (3 females and 3 males) were randomly selected from each replicate at the end of the feeding trial and were used to evaluate the carcass and meat characteristics. The chickens were starved of feed for 16 hours weighed and slaughtered. The slaughtered chickens were properly bled, de-feathered and eviscerated. The internal organs were rapidly removed and weighed. The relative organ weights were calculated by g/% kg body weight (BW). The hot carcasses were weighed to obtain the dressed weight and later chilled before primal cuts were made and the weight taken (Omojola *et al.*, 2004). The results were shown on the Table 5.

The meat yield and quality in experimental lots supplemented with enzymes were attained as high and same level with control group. The differences of meat yields between the experiment groups were not significant ($P > 0.05$).

Internal organs such as heart, liver, spleen, pancreas, proventriculus, caeca and gizzard in both sexes of chicken were measured. The result showed that the ratio of those organs to live weight there no significantly differences ($P > 0.05$) to control diet group. This is indicating that supplemented our *Apsergillus* enzyme preparations could improve live weight but did not show any toxicity to the chickens. This conclusion was supporting by the previous results of studying on toxicity of these fungi, that showed they were safe since did not produce mycotoxins such as aftoflaxin group, ochratoxin A and other (Xuan and Hang, 2006; Ly and Hang, 2006; Cong and Hang, 2005).

These findings were compatible to many results reported so far. Watee Kongbuntad *et al.*, 2006, reported that supplementation of *T. aurantiacus SL* 16 W enzyme to the basal diet of Thai Native chickens improve their FRC and BWG, meanwhile no toxic effect on internal chickens. The same results were reported by Alam *et al.* (2003); Omojola and Adesehinwa, 2007.

Conclusion: Apparently, supplementing our *Aspergillus* spp. enzyme preparations to corn-soy bean based diet could improve performance of Luong Phuong Strain chicken as indicating by markedly body weight increased and FCE decreased. Supplementing *Aspergillus* spp. enzyme preparations did not impose bad effect on meat

quality and internal organs of Luong Phuong Strain chicken as indicating no significantly in ratio of internal organs to live weight of chicken in all groups with or without edition of the enzymes. Addition of the combinations of *Aspergillus* spp. enzyme preparations to diet showed higher efficacy on chickens than Green enzymes (China). This result indicated that our *Aspergillus* spp. enzyme preparations were more effective on local feed components used in Vietnam than imported enzyme.

On the basis of the data obtained, it can be concluded that our *Aspergillus* spp. enzyme preparations in combination was effective preparation for fattening chickens fed with corn/soybean based diets.

Acknowledgment

The authors would like to express our thanks to SIDA/SAREC Project for supporting the research.

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