Journal of Animal and Feed Sciences, 14, 2005, 151–160

Effects of an *Aspergillus niger* mycelium, antibiotic and probiotic on growth performance, P and Ca retention and *Clostridium perfringens* ileal counts in broiler chickens

D. Gogol[1], J. Koreleski[2] and K. Żyła[1,3]

[1]University of Agriculture, Department of Food Biotechnology
29 Listopada 46, 31-425 Kraków, Poland
[2]National Research Institute of Animal Production, Department of Animal Nutrition
32-083 Balice, Poland

(Received 31 March 2004; revised version 4 November 2004; accepted 31 January 2005)

ABSTRACT

A study was conducted to evaluate effects of an *Aspergillus niger* mycelium, antibiotic and probiotic on growth performance, P and Ca retention and *Clostridium perfringens* ileal counts in broiler chickens. Chickens were fed maize-wheat-soyabean meal diets from day 1 to 21. Dietary treatments included: 1. a low phosphorus diet (LP), 0.17% nonphytate phosphorus, 0.6% Ca; 2. LP + virginiamycin; 3. phytase diet (Phyt), LP + 750 phytase units/kg diet; 4. Phyt + virginiamycin; 5. fungal mycelium diet (Myc), LP + 1.5% mycelium; 6. standard phosphorus diet (SP), 0.41% nonphytate phosphorus, 0.87% Ca; 7. SP + virginiamycin; 8. probiotic diet (Pr), SP + probiotic; 9. high phosphorus diet (HP), 0.45% nonphytate phosphorus, 1% Ca; 10. HP + virginiamycin. Each diet was fed to five pens replicates of eight birds each from hatch to 21 day of age.

Chickens receiving Myc, Pr, both HP, and both SP diets performed better than birds fed both LP and both Phyt diets and showed the highest bone mineralization. The lowest intestinal viscosity (1.53 mPa·s) and the highest phosphorus retention (62.1%) was observed in birds fed diet supplemented with fungal mycelium. Birds fed Myc, Pr, both SP and both HP diets showed the highest calcium retention that did not differ from each other. Antibiotic was the most effective in reducing *C. perfringens* counts in ileal digesta. The study confirmed a high efficacy of *A. niger* mycelium used at the level of 1.5% in enhancing growth performance and P retention in chickens.

KEY WORDS: broilers, *Aspergillus niger* mycelium, phytase, probiotic, antibiotic, P, Ca, retention

[3] Corresponding author: e-mail: kzyla@ar.krakow.pl

INTRODUCTION

In order to achieve maximum phytate phosphorus utilization in poultry, different strategies have been undertaken. Turkeys fed low P diets supplemented with enzymic cocktails performed as well as birds receiving control diets recommended by the NRC and retained more P and Ca (Żyła et al., 1996). Turkeys fed diets containing 5% of an industrial waste *Aspergillus niger* mycelium performed even better and retained more P than poults fed the enzymic mixture. The 4% addition of an *A. niger* mycelium biosynthesized on a laboratory scale to the broiler diets resulted in performance, bone mineralization, P and Ca retention superior to values found in birds fed the standard phosphorus diets (Żyła, 2000). In our previous studies (Żyła et al., 1995; Żyła and Gogol, 2002) we have found that the *A. niger* mycelium is high not only in different dephosphorylating activities (phytase, acid phytase, phosphodiesterase), but also in activities that degrade plant cell walls (xylanase, β-glucanase, cellulase, polygalacturonase and acid protease) and reduce intestinal viscosity in broilers. Moreover, preliminary results (Żyła et al., 2000) suggested that mycelial cells or cells fragments might modulate the immune system of birds and influence the intestinal microflora by colonizing the gut and competing with the host microflora.

Clostridium perfringens is regarded as the causative agent of necrotic enteritis (Paulus and Ruckebusch, 1996) and is one of the main factors causing growth depression in poultry (Stutz and Lawton, 1984a). The application of antibiotics as feed additives in poultry production has been limited lately. Feed industry looking for alternative additives is testing different probiotics, prebiotics, acidifiers and new preparations.

The aim of the present study was to evaluate the effectiveness of a mycelial biocatalyst fed to broilers at 1.5% in respect to birds performance, bone mineralization, retention of P and Ca and digesta viscosity. Another purpose of the study was to learn the efficacy of *A. niger* mycelium in lowering ileal *C. perfringens* numbers as compared to virginiamycin and to a probiotic culture.

MATERIAL AND METHODS

Material and diets

The following feed additives were compared: commercial phytase (Natuphos 5000), *Aspergillus niger* mycelium, antibiotic (virginiamycin) and probiotic (Acid-Pack-Four-Way preparation containing living cells of *Lactobacillus acidophilus* and *Streptococcus faecium*). The *A. niger* mycelium was produced at a laboratory scale by a liquid state fermentation technique on a molasses medium. After 4 days of cultivation

fungal mycelium was washed free from the medium, dried by air at 35°C and milled on a grinder. The following diets were formulated: 1. a low phosphorus (LP), 0.17% nonphytate phosphorus, 0.6% Ca; 2. LP + 10 mg virginiamycin/kg diet; 3. phytase diet (Phyt), LP + 750 phytase units/kg diet; 4. Phyt + 10 mg virginiamycin/kg diet; 5. fungal mycelium diet (Myc), LP + 15 g mycelium/kg diet; 6. standard phosphorus diet (SP), 0.41% nonphytate phosphorus, 0.87% Ca; 7. SP + 10 mg virginiamycin/kg diet; 8. probiotic diet (Pr), SP + probiotic added to the drinking water 1.5 ml/L, 0.41% nonphytate phosphorus, 0.87% Ca; 9. high phosphorus diet (HP), 0.45% nonphytate phosphorus, 1% Ca; 10. HP +10 mg virginiamycin/kg diet. The diets 9 and 10 was formulated to have a balanced two-way Anova design (factors: treatment, antibiotic) (Table 1). Commercial phytase, virginiamycin and mycelium were premixed with a small quantity of feed and added to the remaining part of a diet during final mixing.

TABLE 1

Composition and nutrient component of diets[1]

Indices	Diets		
	LP	SP	HP
Ingredient, g/kg			
wheat	316.75	316.75	278.25
maize	250	250	250
soyabean meal	369	369	379
rapeseed oil	48	48	59
limestone	10.5	10.5	9.5
dicalcium phosphate	-	1.35	1.85
NaCl	3	3	3
DL-methionine	1.5	1.5	1.5
vitamin -mineral premix [2]	1.25	1.25	1.25
Calculated			
metabolizable energy, kcal/kg	3000	3000	3000
crude protein, g	220	220	220
nonphytate P, g	1.7	4.1	4.5
P, total, g	4.1	6.5	7.5
Ca, g	6.0	8.7	10.0

[1] the following experimental diets were formulated: 1. a low phosphorus diet (LP); 2. LP + 10 mg virginiamycin/kg diet; 3. phytase diet (Phyt), LP + 750 phytase units/kg diet: one unit of phytase activity was defined as the amount of enzyme required to liberate 1 μM of inorganic phosphorus from 3.5 μM sodium phytate in one min under 40ºC, pH 5.0; 4. Phyt + 10 mg virginiamycin/kg diet; 5. fungal mycelium diet (Myc), LP + 15 g mycelium/kg diet; 6. standard phosphorus diet (SP); 7. SP + 10 mg virginiamycin/kg diet; 8. probiotic diet (Pr), SP + probiotic added to the drinking water 1,5 ml/l; 9. high phosphorus diet (HP), 0.45% nonphytate phosphorus, 1% Ca; 10. HP + 10 mg virginiamycin/kg diet Ingredients were added at the expense of wheat and the metabolizable energy was adjusted by increasing amounts of vegetable oils

[2] supplied per kg of feed: IU: retinyl acetate, 12,000; cholecalciferol, 3,000; mg: dl-α-tocopheryl acetate, 20; menadione sodium bisulphite, 3; thiamin mononitrate, 2; riboflavin, 6; pyridoxine, 2; nicotinic acid, 20; calcium pantothenate, 12; folic acid, 1; choline-HCl, 200; Mn, 65; Zn, 50; Fe, 20; Cu, 6; I, 0.5; Se, 0.1; Co, 0.2; ethoxyquin, 125; diclasuril, 1; μg: cyanocobalamin, 15; biotin, 50

Animals and sample collection

The experiment was conducted on 400 Ross 208 broilers. Day-old chickens were randomly allotted to 10 groups in 5 replication of 8 chickens (4 males and 4 females) each and kept in cages with mesh floors. The birds were housed under standard conditions and fed three weeks *ad libitum* with mash diets (Table 1). Over the entire experimental period feed consumption and chickens growth were measured. On day 21 of age after 4 h of feed deprivation, the birds were weighed, feed intake measured and feeding of the experimental diets resumed.

During the 3rd week (days 15 to 20), a total collection of excreta from each pen was carried out. Excreta were stored in plastic bags at -20^{o}C and then, after thawing were dried in an oven at 50^{o}C to a constant weight. Prior to analysis, excreta were ground to pass a 1 mm sieve. On day 21, three broilers from every replicate were killed by cervical dislocation. Toe samples of three chickens (selected at random) from each replicate were collected for ash analysis. The samples were dried at 100^{o}C for 24 h, weighed, and ashed at 600^{o}C overnight for determination of toe ash. After dissection, the abdominal cavity was opened and the contents of the ileum was collected. Digesta from 3 birds were pooled, then centrifuged at 10.000 g for 10 min and the viscosity of the supernatant was immediately measured on a capillary viscometer. Nine chickens from tested treatments were sacrificed for enumeration of *C. perfringens* in the ilea. The cut section of the ileum, that portion of the small intestine from the yolk stalk to the ileocaecal junction, was ligated with a nylon suture.

Chemical and microbiological assays

Duplicate samples of feed were digested by wet ash procedure, which was validated by including standard reference material 1572 (Citrus leaves) from the National Institute of Standards and Technology (USA). Phosphorus concentration was determined colorimetrically by the molybdeno-vanadate method (AOAC, 1995). Calcium was analysed by flame atomic absorption spectrophotometry. P and Ca retention were calculated relative to P and Ca intake. The quantitative analysis and the typing of the intestine contents for *C. perfringens* were carried out as described by Stutz and Lawton (1984b) and expressed as a decimal logarithm from the most probable counts number (MNP) per 1 g of digesta.

Statistical analysis

Data were analysed statistically by general linear models procedure of Statgraphics Plus for Windows (1996). Mean differences were determined by

Fischer's least significant difference test at P<0.05. Correlation coefficients was calculated and statistical significance determined between body weight gains and *C. perfringens* counts. Two-way Anova (factors: treatment, antibiotic) was performed on data excluding the Myc and Pr groups.

RESULTS

There were significant effects of dietary additions on feed intake, body weight, toe ash, intestinal viscosity, P and Ca retention, and the number of *C. perfringens* (Tables 2 and 3). Chickens fed the low P (LP) diets with and without antibiotic consumed less feed and gained less weight than those assigned to Phyt, Myc, standard phosphorus (SP), Pr and high phosphorus (HP) dietary treatments. Phytase supplementation of LP diet resulted in significant increases in feed intake and body weight gain. Feed intake in birds fed the Myc, SP, Pr,

TABLE 2

Performance and toe ash of broilers fed maize-wheat-soyabean meal diets enriched with phytase, antibiotic, probiotic and fungal mycelium from hatch to 21 d of age[1]

Treatments	Item			
	feed intake g	body weight g	feed efficiency feed: gain, g:g	toe ash, %
LP	667^{a}	630^{a}	1.53	8.22^{a}
LP + antibiotic	720^{a}	593^{a}	1.47	8.60^{ab}
Phyt	872^{b}	724^{b}	1.48	10.17^{c}
Phyt + antibiotic	883^{bc}	730^{b}	1.47	9.67^{bc}
Myc	935^{bcd}	810^{cd}	1.48	12.74^{d}
SP	915^{bcd}	749^{bc}	1.42	12.43^{d}
SP + antibiotic	964^{d}	839^{d}	1.47	12.60^{d}
Pr	904^{bcd}	704^{b}	1.42	11.88^{d}
HP	946^{cd}	874^{d}	1.43	12.26^{d}
HP + antibiotic	940^{bcd}	868^{d}	1.42	12.19^{d}
SEM	24.37	24.42	0.025	0.39
P-value				
antibiotic[2]	0.145	0.443	0.504	0.991
treatment	0.001	0.001	0.005	0.001
antibiotic × treatment[2]	0.582	0.065	0.113	0.734

^{a,b} values within the same column with different letters differ significantly (P<0.05)

[1] treatments were as followed: 1. a low phosphorus diet (LP); 2. LP + 10 mg virginiamycin /kg diet; 3. phytase diet (Phyt), LP + 750 phytase units/kg diet; 4. Phyt + 10 mg virginiamycin /kg diet; 5. fungal mycelium diet (Myc), LP + 15 g mycelium/kg diet; 6. standard phosphorus diet (SP), 0.41% nonphytate phosphorus, 0.87% Ca; 7. SP + 10 mg virginiamycin/kg diet; 8. probiotic diet (Pr), SP + probiotic added to the drinking water 1.5 ml/l; 9. high phosphorus diet (HP); 10. HP + 10 mg virginiamycin/kg diet

[2] two-way Anova (factors: treatment, antibiotic) was performed on data excluding the Myc and Pr diets

TABLE 3

Digesta viscosities, P and Ca retention and *Clostridium perfringens* counts of broilers fed maize-wheat-soyabean meal diets enriched with phytase, antibiotic, probiotic and fungal mycelium from hatch to 21 d of age[1]

Treatments	Item			
	digesta viscosity mPa·s	P retention %	Ca retention %	*C. perfringens* $\log_{10}$cfu/g digesta
LP	2.18^{f}	49.68^{ab}	44.95^{bcd}	-
LP + antibiotic	1.81^{bc}	46.49^{abc}	43.8^{abc}	-
Phyt	1.78^{b}	43.00^{ab}	38.08^{ab}	-
Phyt + antibiotic	1.56^{a}	44.60^{ab}	35.60^{a}	-
Myc	1.53^{a}	62.11^{d}	52.13^{cde}	1.37^{b}
SP	1.85^{cd}	53.13^{c}	57.49^{e}	2.04^{c}
SP + antibiotic	2.12^{e}	45.03^{abc}	51.92^{cde}	0.93^{a}
Pr	1.80^{b}	50.65^{bc}	53.87^{de}	1.45^{b}
HP	1.89^{d}	42.90^{ab}	53.64^{de}	-
HP + antibiotic	1.87^{d}	40.97^{a}	53.06^{cde}	-
SEM	0.014	2.88	3.21	0.096
P-value				
antibiotic[2]	0.001	0.164	0.303	-
treatment	0.001	0.060	0.001	0.001
antibiotic × treatment[2]	0.001	0.418	0.869	-

[a,b] values within the same column with different letters differ significantly ($P<0.05$)

[1] treatments were as followed: 1. a low phosphorus diet (LP); 2. LP + 10 mg virginiamycin/kg diet; 3. phytase diet (Phyt), LP + 750 phytase units/kg diet; 4. Phyt + 10 mg virginiamycin/kg diet; 5. fungal mycelium diet (Myc), LP + 15 g mycelium/kg diet; 6. standard phosphorus diet (SP); 7. SP + 10 mg virginiamycin/kg diet; 8. probiotic diet (Pr), SP + probiotic added to the drinking water 1,5 ml/L; 9. high phosphorus diet (HP), 0.45% nonphytate phosphorus, 1% Ca; 10. HP + 10 mg virginiamycin/kg diet

[2] two-way Anova (factors: treatment, antibiotic) was performed on data excluding the Myc and Pr diets

HP diets were similar but significantly higher than birds fed the LP and Phyt diets. The influence of phytase, probiotic and fungal mycelium on body weight parallelled that of feed intake, except the standard phosphorus group, where the antibiotic addition significantly increased the body weight, but did not affect feed intake. There were no significant differences among treatments in respect to feed conversion ratio. The phytase addition to the LP diet, as well as to the LP diet containing antibiotic, resulted in significant increase in toe ash. Chickens receiving the Myc diet showed the highest percentage of ash in the toe (12.7%). Toe ash found in birds of the Myc, SP, Pr, HP diets were similar but significantly higher than in birds fed the LP and Phyt diets. The highest intestinal viscosities were found in birds fed the low phosphorus diet without antibiotic (2.18 mPa·s). Supplementation of antibiotic to the LP diet significantly reduced digesta viscosity. The same downtrend was observed in groups receiving phytase without and with

antibiotic. However, in the standard phosphorus diet that did not contain antibiotic lower viscosity (1.85 mPa·s) was found than in the diet enriched with antibiotic (2.12 mPa·s). The lowest intestinal viscosity was observed in birds consuming diet supplemented with fungal mycelium (1.53 mPa·s). The highest P retention was observed in birds fed Myc diet, significantly lower values were found in the SP group without antibiotic and the Pr group. Ca retention in Myc, Pr, both SP and both HP diets was the highest and did not differ from each other. The numbers of *C. perfringens*, for the birds that did not receive antibiotic (2.04 $\log_{10}$cfu/g digesta), were higher than *Clostridium* counts in other tested dietary treatments. Addition of fungal mycelium or probiotic to the diets significantly reduced the population of *C. perfringens* in the ileum (1.37 and 1.45 $\log_{10}$cfu/g digesta, respectively) but was less effective than virginiamycin (0.93 $\log_{10}$cfu/g digesta). Two-way Anova (Tables 2 and 3) demonstrated that the influence of treatment on the growth performance parameters, toe ash, intestinal viscosity and Ca retention was significant ($P<0.05$). There was no influence of the treatments on P retention, however. Antibiotic did not significantly influence the parameters tested except for digesta viscosity. Linear regression analysis revealed inverse correlation ($r = -0.307$), at $P = 0.069$ between body weight of broilers and *C. perfringens* counts in the ilea. The reciprocal-X model produced even stronger relationship ($r = -0.381$; $P=0.022$).

DISCUSSION

In the study presented here low phosphorus diets were supplemented with commercial phytase or with *A. niger* mycelium and fed to growing chickens. Phytase addition to the low phosphorus diet improved both performance and bone mineralization of chickens and significantly decreased digesta viscosity. Birds fed diets supplemented with phytase had higher P and Ca retention than birds fed low phosphorus diets. This finding is consistent with results from our previous studies (Żyła et al., 1996, 2000). The experiment was designed to find whether there are any interactions between antibiotic and phytase added to diets in enhancing broilers growth performance. Antibiotic did not affect chicken performance except for the standard phosphorus group where it increased significantly body weight. Engberg et al. (2000) observed significant growth promoting effect in chickens fed diet supplemented with combination of antibiotics: zinc bacitracin with salinomycin. Studies conducted by Mohan et al. (1996) proved that broiler chickens fed probiotic and antibiotic supplemented diets for four weeks had the highest weight gain, followed by those receiving antibiotic alone, probiotic alone and controls. In our experiment antibiotic added to the low phosphorus

diet and to the phytase diet caused significant decrease in digesta viscosity in relation to diets without antibiotic. However, in the case of standard phosphorus group antibiotic increased intestinal viscosity. Moreover, in birds fed the two HP diets no significant differences in digesta viscosity were found. It seems possible therefore that interactions between antibiotic and phytase are related to the level of available phosphorus and calcium in the diet.

A. niger mycelium fed at 1.5% proved its highly effective dephosphorylating abilities (Żyła et al., 1996, 2000). Chickens fed mycelium supplemented diet performed similarly to chickens fed the standard phosphorus and HP diets. They showed enhanced bone mineralization, and high retention of P and Ca. The mycelium addition to a diet caused 28% reduction in digesta viscosity as compared to the standard phosphorus diet containing antibiotic. In the previous study the same fungal mycelium used at 4% caused 40% reduction of intestinal viscosity (Żyła, 2000). Several preparations have been tested to find their efficacies in enhancing poultry performance and reducing *C. perfringens* counts in the ileum. Nurit et al. (2001) checked non-antibiotic growth promoters Crina® Poultry and More Yeast 100E against antibiotic growth promoter virginiamycin used at level of 20 ppm. Both tested preparations improved feed conversion and slightly increased body weight of chickens. Crina® Poultry preparation, a blend of essential oil compounds, improved feed efficiency and reduced digesta viscosity (Francesch et al., 1999). Moreover the preparation prevented the colonization of the intestine of broilers by *C. perfringens* (Williams and Losa, 2001a). Acid Lac® Dry, a mixture of organic acids, was found to numerically reduce *C. perfringens* counts but not to the same extend as the antibiotic (Van Campenhout et al., 2001). In our study chickens fed diets supplemented with fungal mycelium and probiotic had reduced number of *C. perfringens*, but still the numbers were higher than in birds receiving 10 ppm virginiamycin. In the studies of Stutz and Lawton (1984a) virginiamycin supplemented at levels of 55 and 16.5 ppm significantly improved weight gain and decreased *C. perfringens* counts. Body weight gain of birds fed SP diet supplemented with antibiotic as compared to a SP diet without antibiotic was significantly increased. In other dietary treatments no such a significant effect of the antibiotic was found. The inverse correlation between body weight of broilers and ileal *C. perfringens* counts observed in our study is consistent with observations of Stutz and Lawton (1984a). They found highly significant inverse correlations ($r=-0.879$; $P<0.01$) in broilers between weight gain and *C. perfringens* counts.

Results from the studies on the effects of probiotic cultures on animal production are often contradictory. The beneficial effect of probiotic cultures on growth performance and intestinal microbiota have been documented (Tortureo, 1973; Pascual et al., 1999; Jin et al., 2000). However, there have also been reports

of no significant differences in weight gains of chickens fed diets with or without *Lactobacillus* cultures (Watkins and Kratzer, 1984; Maiolino et al., 1992). We observed no improvements neither in performance, toe ash nor in P and Ca retention in broilers fed probiotic supplemented diet as compared to the standard phosphorus diet. Only digesta viscosity of birds receiving probiotic were lower than in birds fed the standard phosphorus diet.

The study presented here confirmed a high efficacy of a novel, not registered feed additive - *A. niger* mycelium, fed at a level of 1.5% in enhancing growth performance of chickens. Additionally, it effectively inhibited growth of *C. perfringens* thus preventing growth depression. Since *A. niger* mycelium caused reduction in intestinal viscosity to a much higher extent than phytase, probiotic or antibiotic and was found to be high in xylanase, β-glucanase, cellulase activities (data not presented) it could probably replace NSP degrading enzymes and increase nutrient digestibility. It needs to be emphasized, however, that *A. niger* mycelium was added to the low phosphorus control diet, whereas other experimental diets subjected to microbiological analysis contained the standard phosphorus level. Concluding, the *A. niger* mycelium seems to be a highly effective biocatalyst with a possible probiotic role.

REFERENCES

AOAC, 1995. Official Methods of Analysis, Association of Official Analytical Chemists. 16th Edition. Washington, DC

Engberg R.M., Hedemann M.S., Leser T.D., Jensen B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poultry Sci. 79, 1311-1319

Francesch M., Brufau J., Badiola I., Llaurado Ll., Llach J.R., 1999. Effects on the essential oil blend crina HC in feed on broiler performance and digesta viscosity. Proceedings of 12th European Symposium on Poultry Nutrition. Veldhoven (The Netherlands), p. 128

Jin L.Z., Ho Y.W., Abdullah N., Jalaludin S., 2000. Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* cultures. Poultry Sci. 79, 886-891

Maiolino R., Fioretti A., Menna L.F., Meo C., 1992. Research on the efficiency of probiotics in diets for broiler chickens. Nutr. Abstr. Rev., Ser. B 62, 482

Mohan B., Kadirvel R., Natarajan A., Bhaskaran M., 1996. Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. Brit. Poultry Sci. 37, 395-401

Nurit S., Rulf Y., Miller N., Schatz A., Hoyzman A., 2001. Probiotics versus antibiotics as growth promoters in broiler chickens. Proceedings of 13th European Symposium on Poultry Nutrition. Blankenberge (Belgium), p. 135

Pascual M., Hugas M., Badiola J.I., Monfort J.M., Garriga M., 1999. *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteriditis* colonization in chickens. Appl. Environ. Microbiol. 65, 4981-4986

Paulus C., Ruckebusch J.P., 1996. Necrotic enteritis (NE). Zootec. Int. XIX, 6, 40

Statgraphics Plus for Windows, 1996. Advanced Regression. Manugistics, Inc. Rockville, MA

Stutz M.W., Lawton G.C., 1984a. Effects of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens* and ileal weight of broiler chicks. Poultry Sci. 63, 2036-2042

Stutz M.W., Lawton G.C., 1984b. The iron milk most probable number method for enumeration of *Clostridium perfringens* in the diet and the intestine of the chick. Poultry Sci. 63, 2041-2046

Tortureo F., 1973. Influence of implantation of *Lactobacillus acidophilus* in chicks on the growth, feed conversion, malabsorbtion of fats syndrome and intestinal flora. Poultry Sci. 52, 197-203

Van Campenhout L., Van Hemel J., Vandenkerckhove J., Mollen K., Sas B., 2001. Performance of an alternative to antibiotics in broilers with high intestinal counts of *Clostridium perfringens*. Proceedings of 13th European Symposium on Poultry Nutrition. Blankenberge (Belgium), pp. 127-128

Watkins B.A., Kratzer F.H., 1984. Drinking water treatment with commercial preparation of a concentrated *Lactobacillus* culture for broiler chickens. Poultry Sci. 63, 1671-1673

Williams P., Losa R., 2001a. Prevention of colonisation of *Clostridium perfringens* in broilers intestine by essential oils. Proceedings of 13th European Symposium on Poultry Nutrition. Blankenberge (Belgium), p. 133

Williams P., Losa R., 2001b. The use of essential oils in poultry nutrition. Int. Poultry Prod. 9 (2), 11-13

Żyła K., 2000. A novel biocatalyst for dephosphorylation of poultry feeds. In: 3rd European Symposium on Feed Enzymes. Abstracts. Noordwijkerhout (The Netherlands), p. 85

Żyła K., Gogol D., 2002. In vitro efficacies of phosphorolytic enzymes synthesized in mycelial cells of *Aspergillus niger* AbZ4 grown by a liquid surface fermentation. J. Agr. Food Chem. 50, 899-905

Żyła K., Ledoux D.R., Garcia A., Veum T.L., 1995. An *in vitro* procedure for studying enzymic dephosphorylation of phytate in maize-soya bean feeds for turkey poults. Brit. J. Nutr. 74, 3-17

Żyła K., Ledoux D.R., Kujawski M., Veum T. L., 1996. The efficacy of an enzymic cocktail and a fungal mycelium in dephosphorylating corn-soybean meal-based feeds fed to growing turkeys. Poultry Sci. 75, 381-387

Żyła K., Wikiera A., Koreleski J., Świątkiewicz S., Piironen J., Ledoux D.R., 2000. Comparison of the efficacies of a novel *Aspergilllus niger* mycelium with separate and combined effectiveness of phytase, acid phosphatase, and pectinase in dephosphorylation of wheat-based feeds fed to growing broilers. Poultry Sci. 79, 1434-1443

STRESZCZENIE

Wpływ grzybni *Aspergillus niger*, antybiotyku i probiotyku na wzrost oraz liczbę bakterii *Clostridium perfringens* w treści jelitowej kurcząt brojlerów

Kurczęta brojlery karmiono paszą pszenno-kukurydzianą z dodatkiem śruty sojowej. Stosowano następujące diety: 1. o niskiej zawartości fosforu (LP), 0,17% fosforu niefitynianowego, 0,6% Ca; 2. LP + virginiamycyna; 3. LP + 750 jednostek fitazy/kg paszy (Phyt); 4. Phyt + virginiamycyna; 5. LP + 1,5% dodatek grzybni *A. niger* (Myc); 6. o standardowej zawartości fosforu (SP), 0,41% fosforu niefitynianowego, 0,87% Ca; 7. SP + virginiamycyna; 8. SP + probiotyk (Pr); 9. o wysokiej zawartości fosforu (HP), 0,45% fosforu niefitynianowego, 1% Ca; 10. HP + virginiamycyna. W grupach ptaków karmionych dietą Myc, Pr, obydwóch grupach HP oraz obydwóch grupach o standardowej zawartości fosforu obserwowano lepsze parametry wzrostu i odchowu niż w pozostałych grupach oraz lepszą mineralizację kośćca. Najmniejszą lepkość treści jelitowej oraz największą retencję fosforu stwierdzono w grupach ptaków otrzymujących paszę z dodatkiem grzybni. Ptaki otrzymujące paszę z dodatkiem grzybni oraz paszę z dodatkiem probiotyku wykazywały znacząco niższą liczbę bakterii *C. perfringens* w treści jelitowej niż ptaki otrzymujące paszę bez jakiegokolwiek dodatku, ale wyższą niż w grupie otrzymującej antybiotyk. Doświadczenie potwierdziło wysoką efektywność 1,5% dodatku grzybni *A. niger* do paszy w polepszaniu wskaźników wzrostu i odchowu kurcząt oraz zwiększeniu retencji fosforu.