Emir. J. Food Agric. 2013. 25 (7): 549-554
doi: 10.9755/ejfa.v25i7.12364
http://www.ejfa.info/

ANIMAL SCIENCE

Effects of a phytogenic feed additive on growth performance, selected blood criteria and jejunal morphology in broiler chickens

Abdulkarim A. Amad[1*], K. R. Wendler[2] and J. Zentek[3]

[1]*Department of Animal Production, Faculty of Agriculture and Veterinary, Thamar University, Dhamar, Yemen*
[2]*Delacon Biotechnik, Steyregg, Austria*
[3]*Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany*

Abstract

The study was conducted to examine the effects of a phytogenic feed additive (PFA; BIOSTRONG® 510) on growth performance, selected blood parameters and jejunal morphology in broiler chickens. The PFA consists of a mixture of essential oils with thymol and anethole as leading active substances, as well as different herbs and spices. A total of 264 1-d-old Cobb male broilers were randomly allocated to 2 dietary treatments, with 6 replicates per treatment and 22 birds each replicate. The experiment lasted 42 days. The dietary treatments were a starter and grower diet without feed additives (control), or the diets supplemented with 150 mg/kg of the PFA. Body weight and feed intake were not significantly influenced by PFA feeding compared with the control during all experimental periods. During the grower phase (22 - 42 d of age) and during the whole period (1 - 42 d of age) the PFA significantly improved ($P<0.05$) feed conversion ratio. Serum total protein, albumin, total cholesterol and leucocytes were increased by PFA feeding. Furthermore, villus height to crypt depth ratio in the jejunum was increased by PFA feeding. In conclusion, the results of this study show that inclusion of PFA lead to morphological changes in the jejunum, which might influence nutrient absorption and thus, improved FCR.

Key words: Broiler chicken, Performance, Blood parameters, Jejunal morphology

Introduction

The ban of antibiotic growth promoters (AGP) in poultry feeds intensified the search for alternatives improving the health and productivity of broiler chickens (Barreto et al., 2008). Such alternatives are probiotics, prebiotics and organic acids, which are added to the feed (Huyghebaert et al., 2011). Also phytogenic feed additives (PFA) were shown to enhance performance in AGP-free livestock production (Alçiçek et al., 2003; Steiner, 2009). Phytogenic feed additives consist of a broad variety of substances, mainly extracts from plant materials, such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Burt, 2004). The active molecules include many different secondary plant metabolites, resulting in a broad range of physiological effects, like secretolytic and spasmolytic, or immune-stimulative effects (Lee et al., 2004a).

PFA are generally recognized as natural feed additives and safe to the animal. However, results of studies concerning the use of PFA in broiler nutrition are inconsistent (Windisch et al., 2008). Some authors stated significant improvement of broiler performance (Ertas et al., 2005; Cross et al., 2007), whereas others reported no effects on BW gain and feed intake (Lee et al., 2003; Jamroz et al., 2005; Nasir and Grashorn, 2010) or feed conversion ratio (Ocak et al., 2008). These discrepancies may be due to numerous factors such as type and parts of plants used, their physical properties, time of harvest, the preparation method of PFA and their compatibility with other feed components (Jang et al., 2007). Furthermore, the mode of action of these additives is not fully clarified yet and in vivo studies are limited. Plant extracts have been shown to influence digestion and secretion of digestive enzymes (Platel and Srinivasan, 2000; Williams and Losa, 2001) to increase absorption of micronutrients (Usha et al., 2010) and to exhibit antibacterial, antiviral and

Received 14 May 2012; Revised 29 December 2012; Accepted 08 January 2013; Published Online 01 May 2013

*Corresponding Author

Abdulkarim A. Amad
Department of Animal Production, Faculty of Agriculture and Veterinary, Thamar University, Dhamar, Yemen

Email: al_absie@yahoo.com

antioxidant activities (Brenes and Roura, 2010). However, only few studies investigated the effects of PFA on intestinal morphology in broiler chickens. Therefore, the aim of the present study was to examine the effect of a commercial PFA on performance, intestinal morphology and selected blood parameters in broiler chickens.

Materials and Methods

Two hundred sixty four 1-d-old male Cobb chickens were weighed and randomly allotted to 2 experimental treatments. Each treatment consisted of 6 replicates with 22 birds per replicate. The dietary treatments were a starter and grower basal diet without any feed additives used as a control or the basal diets supplemented with 150 mg/kg of a commercial PFA (BIOSTRONG® 510, Delacon, Steyregg, Austria) used as experimental diets. The PFA consisted of a mixture of essential oils, with thymol and anethole as leading active substances, as well as different herbs and spices. The compositions of the basal diets are shown in Table 1. Control and experimental diets were formulated to be iso-nutritive and to meet the requirements for broiler chickens during starter and grower phase. Feed and water were available *ad libitum*. Feed consumption and BW of the birds were recorded weekly and were used to calculate broiler performance (weight gain, feed intake, feed conversion ratio).

Blood samples were taken at 35 d of age from the brachial vein from 12 birds per treatment group (2 birds per pen). Blood samples were separated by centrifuge, and serum was analyzed for glucose, total cholesterol, triglycerides, albumin and total protein using commercial test kits. Blood cell counts were determined by commercial cell counter.

At 42 d of age, 9 birds per treatment were slaughtered and the jejunum was removed for assessment of tissue morphology. The tissue samples were taken from the middle part of the jejunum (from the entrance of bile duct to Meckel's diverticulum, pieces about 5 cm in length), carefully cleansed and then fixed in 4% buffered formalin for 2 days. The further processing consisted of serial dehydration in PBS and graded ethanol solutions, clearing with xylene and embedding in paraffin. Sections of 5 µm were prepared and placed on glass slides. Tissue samples were deparaffinised, rehydrated and stained with haematoxylin and eosin. Villus heights and crypt depths were examined with a photomicroscope (Photomikroskop III, Carl Zeiss, Oberkochern, Germany) fitted with a digital camera (MikroCam 3 MP, Bresser, Rhede, Germany) and images were analysed using image analysis software (Image software Bresser MikroCamLab Mikroskopie). A total of 16 intact well-oriented villus-crypt units were randomly selected at each tissue sample. Villus height was measured from the tip of the villus to the villus-crypt junction, and crypt depth was defined as the depth of the invagination between two villi.

Experimental data were analyzed by ANOVA using SPSS v. 17.0 (Statistical Packages for the Social Sciences, released August 23, 2008). Data was tested for homogeneity of variances, and comparison of means was performed by Tukey test. The significance level was set at $p < 0.05$.

Results

During the whole experiment, birds were healthy and the mortality was below 1%. Performance data for broiler chickens during the starter and grower phase and for the total experimental period are summarized in Table 2.

Table 1. Ingredients and nutrient composition of experimental diets.

Ingredients (%)	Starter 1 - 21d	Finisher 22 - 42 d
Soybean meal (48% CP)	34.50	31.80
Maize	28.46	29.47
Wheat	24.69	24.29
Soy oil	6.80	9.35
Limestone	1.80	1.51
Monocalcium-phosphate	1.40	1.35
Premix*	1.20	1.20
Chromium oxide	0.50	0.50
DL-Methionine	0.30	0.29
L-Lysine	0.25	0.14
Additives**	0.10	0.15
	100%	100%
Chemical composition (calculated)		
AMEn, (MJ/kg)	12.59	13.29
Crude protein(%)	22.89	21.50
Crude fibre (%)	2.39	2.32
Lysine (%)	1.43	1.26
Methionine (%)	0.64	0.61
Methionine + Cystein (%)	1.02	0.98
Calcium (%)	1.03	0.90
Total Phosphorus (%)	0.70	0.68

*Supplied per kg of diet: 4000 IU vitamin A (retinyl acetate); 400 IU cholecalciferol; 80 mg (α-tocopherole acetate); 3 mg vitamin K3 (menadione); 2.5 mg thiamin ; 2.5 mg riboflavin; 25 mg nicotinic acid; 4 mg pyridoxine; 0.02 mg cobalamin; 0.3 mg biotin; 10 mg calcium pantothenate acid; 1 mg folic acid; 800 mg choline chloride; 50 mg Zn (Zinc oxide); 20 mg Fe (Iron carbonate); 60 mg Mn (manganese oxide); 12 mg Cu (copper sulfate-pentahydrate); 0.45 mg J (calcium iodate; 0.30 mg Co (cobalt- (II)-sulfate-heptahydrate); 0.35 mg Se (sodium selenite); 1.3 g Na (sodium chloride); 0.55 g Mg (magnesium oxide) ; ** Additives: Control = without active substances (PFA); Treatment = Biostrong® 150 mg/kg feed.

For all experimental periods, there was no effect (P>0.05) of the PFA on BWG and feed intake. Feed conversion ratio was significantly improved (P<0.05) in the grower phase (22 - 42 d of age) as well as during the whole experimental period (1- 42 d of age) due to PFA supplementation.

Villus heights, crypt depths and villus height: crypt depth ratios of jejunal tissue samples are presented in Table 4. In broilers fed diets supplemented with PFA, there were no differences between the villus height and crypt depth could be observed compared to the control (Figure 1). Villus height: crypt depth ratio was significantly (P<0.05) increased in birds fed PFA compared to the control.

Effect of the phytogenic feed additive on some blood parameters in broilers were summarized in Table 3. Serum total protein, albumin, cholesterol and leucocytes were increased (P<0.05), while serum glucose, triglycerides, hemoglobin and erythrocytes were not affected by PFA feeding.

Table 2. Effect of the phytogenic feed additive on broiler performance during the starter and grower period (means ± SD).

Period	Treatments		*P-value*
	Control	PFA (150 mg/kg)	
Average body weight gain (g)			
1 - 21 d	797 ±67	813 ±28	0.62
22 - 42 d	2108 ±102	2144 ±150	0.64
1 - 42 d	2906 ±134	2956 ±173	0.58
Average feed intake (g)			
1 - 21 d	1124 ±108	1106 ±99	0.76
22 - 42 d	3332 ±130	3259 ±211	0.49
1 - 42 d	4456 ±202	4365 ±287	0.54
Feed conversion ratio (g/g)			
1 - 21 d	1.41 ±0.07	1.36 ±0.08	0.295
22 - 42 d	1.58^{a} ±0.04	1.52^{b} ±0.03	0.013
1 - 42 d	1.53^{a} ±0.04	1.48^{b} ±0.02	0.011

ab Values with different superscript within lines differ significantly (*P*<0.05)

Table 3. Effect of the phytogenic feed additive on some blood parameters in broilers (35^{th} d of age; means ± SD).

Parameters	Treatments		*P-value*
	Control	PFA (150 mg/kg)	
Serum total protein (g/l)	27.03^{a} ±3.65	30.64^{b} ±1.38	<0.004
Serum albumin (g/l)	11.67^{a} ±2.15	14.22^{b} ±0.87	0.001
Serum glucose (mmol/l)	14.22 ±1.49	14.99 ±0.60	0.11
Serum Cholesterol (mmol/l)	3.00^{a} ±0.65	3.84^{b} ±0.22	<0.001
Triglycerides (mmol/l)	1.33 ±1.23	1.86 ±0.71	0.21
Erythrocytes (Tpt/l)	2.27 ±0.10	2.34 ±0.27	0.31
Leucocytes (Tpt/l)	175.8^{a} ±2.4	183.5^{b} ±9.5	0.012
Haemoglobin (mmol/l)	4.94 ±0.25	5.22 ±0.48	0.09

ab Values with different superscript within lines differ significantly (*P*<0.05)

Table 4. Effect of the phytogenic feed additive on histomorphological parameters of the jejunum in broilers (means ± SD).

Parameters	Treatments		*P- value*
	Control	PFA (150 mg/kg)	
Villus height (µm)	1447±125.4	1569±120.9	0.068
Crypt depth (µm)	199±21.0	179±18.6	0.061
Villus height:crypt depth	7.3^{a}±0.8	8.8^{b}±0.9	0.004

ab Values with different superscript within lines differ significantly (*P*<0.05)

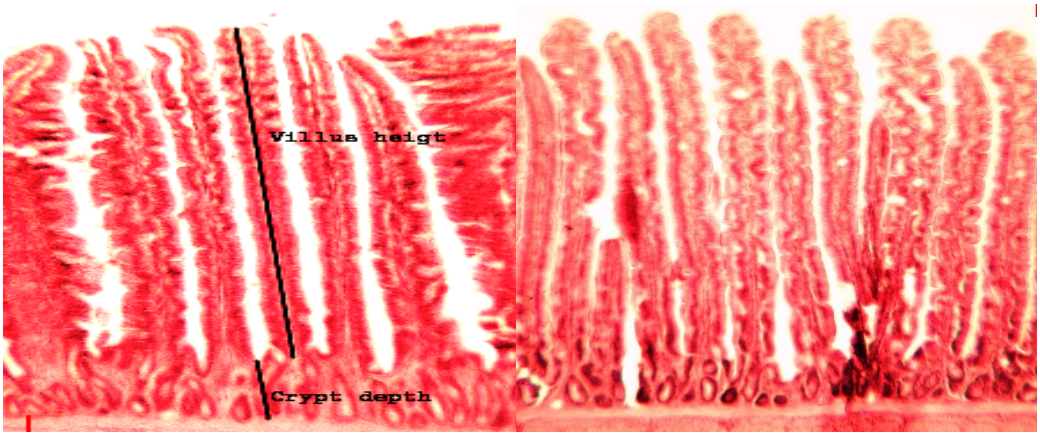

Figure 1. Villus height and crypt depth in the jejunum of broilers fed diets with or without the phytogenic feed additive at 42 days of age, left control, right phytogenic feed additive.

Discussion

In the present study, there was no effect of the tested PFA on BW and feed intake in broilers. These results are consistent with the studies of Jang et al. (2007) and Erdogan et al. (2010), who did not find effects of different phytogenic compounds on broiler growth performance. However, FCR was significantly improved in the present study. Consistently, Jamroz et al. (2005) reported improved FCR due to the addition of a plant extract, containing cinnamaldehyde, carvacrol and capsaicin, to a maize or wheat and barley based diet by 4.1 or 2.0%, respectively, whereas BW was not affected by treatment. Al-Kassie (2009) showed that the addition of 200 ppm oil extract derived from thyme and cinnamon to broiler diets significantly improved BW gain and FCR during a growing period of 6 weeks and Ocak et al. (2008) reported higher ($P< 0.05$) BW at 21 and 42 d of age as well as higher ($P< 0.05$) BW gain from 7 to 35 days of age in broilers fed peppermint and thyme compared to the controls. The above mentioned studies, reporting improved FCR, have in common, that the added substances mainly consist of essential oils. Essential oils exert antimicrobial activity in the digestive tract of animals (Lee et al., 2004; Al-Kassie, 2010). It is hypothesized that gut microflora reduces nutrients available to the animal by enforcing the intestinal cell turnover and thereby increasing the intestinal requirement for nutrients to maintain tissue integrity. Moreover, intestinal microflora and epithelial cells have to compete for nutrients (Dibner and Richards, 2005; Lan et al., 2005). By a reduction of intestinal microflora, essential oils may lead to moderate cell turnover, to decreased intestinal nutrient requirements and to less competition for available nutrients. As a consequence, FCR is improved because more nutrients are used for BW gain instead of tissue maintenance or microbial growth.

The increased nutrient supply for growth is reflected in enhanced nutrient transport in the blood. For example, Ghazalah and Ali (2008) observed higher levels of total protein, albumin and globulin in the blood serum of birds when fed 0.5% rosemary leaves. Similar results were obtained in the present study, where higher contents of total protein and albumin in the blood serum of PFA fed animals may also indicate enhanced nutrient supply and transport. Furthermore, Tekeli et al. (2006) reported increased blood glucose concentration by Zingiber officinale supplementation and increased triglyceride concentration by both Zingiber officinale and Syzygium Aromaticum supplementation. In the present study, there was no effect of PFA on blood glucose and triglyceride concentrations. Calislar et al. (2009) found effects neither on triglycerides nor on blood cholesterol levels by application of a PFA containing Origanum vulgare ssp. hirtum extract. In general, essential oils are more often associated with hypocholesterolemic properties (Lee et al., 2004). In contrast, in the present study chicken fed the PFA showed higher serum cholesterol concentrations compared to the control animals. This discrepancy may be due to the combination of essential oils and pungent substances which were also included in the used PFA. Pungent substances increase digestive secretions including enzymes and bile. The higher serum cholesterol content, which was observed in the PFA fed animals of the present study, may be the result of an increased lipid digestibility due to a higher secretion of bile and digestive enzymes. The improvement of FCR as

well as the increase of blood nutrient concentrations supports this assumption, although it remains speculative as nutrient digestibilities have not been investigated in the present study.

Regarding the intestinal morphology, heavier chickens are generally associated with longer villi, greater villus width and higher villus surface area as compared to lighter ones (Adibmoradi et al., 2006; Incharoen et al., 2010). In the present study there were insignificantly increased villus heights and decreased crypt depths in the jejunum of birds receiving 150 mg/kg PFA compared to the control. Accordingly, the villus height: crypt depth ratio was significantly higher in the birds fed the PFA compared to the control. These findings were consistent to Adibmoradi et al. (2006) who reported that jejunal villus height was increased whereas crypt depths were decreased, leading to increased villus height: crypt depth ratio in birds fed graded levels of garlic meal. It has been suggested that longer villi would result in an increased surface area and higher absorption of available nutrients (Caspary, 1992; Yasar and Forbes, 1999). A higher absorptive capacity of the intestine of PFA fed animals is also supported by higher blood nutrient concentrations of those animals, as observed in the present study. However, although Jamroz et al. (2006) reported similar improvement of FCR due to the supplementation with a plant extract containing carvacrol, cinnamaldehyde and capsicum oleoresin, the authors could not find an effect of the plant extract on intestinal morphology in broilers at 42 d of age. Thus, for PFA containing essential oils as well as pungent substances further, eventually synergistic, effects or other modes of action cannot be ruled out.

Conclusion

In the present study, the addition of the phytogenic feed additive BIOSTRONG® 510 to broiler diets significantly improved feed conversion ratio. The observed improvement of feed conversion ratio may be caused by a combination of different effects, including enhancement of digestive secretions, antimicrobial effects of essential oils as well as enlargement of intestinal absorptive surface. However, further studies are necessary to evaluate the effects of various substances present in phytogenic feed additives and to clarify their specific modes of action.

References

Alçiçek, A., M. Bozkurt and M. Çabuk. 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. S. Afr. J. Ani. Sci. 33:89-94.

Al-Kassie, G. A. M. 2009. Influence of two plant extracts derived from thyme and cinnamon on broiler performance. Pak. Vet. J. 29: 69-173.

Al-Kassie, G. A. M. 2010. The Effect of Thyme and Cinnamon on the Microbial Balance in Gastro Intestinal Tract on Broiler Chicks. Int. J. Poult. Sci. 9:495-498.

Adibmoradi, M., B. Navidshad, J. Seifdavati and M. Royan. 2006. Effect of dietary garlic meal on histological structure of small intestinal in broiler chickens. J. Poult. Sci. 43:378–383.

Barreto, M. S. R., J. F. M. Menten, A. M. C. Racanicci, P. W. Z. Pereira and P. V. Rizzo. 2008. Plant Extracts used as Growth Promoters in Broilers. Bra. J. Poult. Sci. 10:109-115.

Brenes, A. and E. Roura. 2010. Essential oils in poultry nutrition: Main effects and modes of action. Anim. Feed Sci. Techn. 158: 1–14.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods - a review. Int. J. Food Microb. 94:223-253.

Calislar, S., I. Gemci and A. Karnalak. 2009. Effects of Oregano-Stim® on broiler chick performance and some blood parameters. J. Anim. Vet. Adv. 8:2617-2620.

Caspary, W. F. 1992. Physiology and pathphysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299S-308S.

Cross, D. E., R. M. Mcdevitt, K. Hillman and T. Acamovic. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br. Poult. Sci. 48:496–506.

Dibner, J. J. and J.D. Richards. 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci. 84:634-643.

Erdogan, Z. S., S. Erdogan, O. Aslantas and S. C. Elik. 2010. Effects of dietary supplementation of synbiotics and phytobiotics on performance, caecal coliform population and some oxidant/antioxidant parameters of broilers. J. Anim. Phys. Anim. Nutr. 94:40-48.

Ertas, O. N., T Güler, M. Çiftçi, B. Dalkiliçand Ü. G. Simsek. 2005. The effect of an essential oil

mix derived from oregano, clove and anise on broiler performance. Int. J. Poult. Sci. 4:879-884.

Incharoen, T., K. Yamauchi, N. Thongwittaya. 2010. Intestinal villus histological alterations in broilers fed dietary dried fermented ginger. J. Anim. Phys Anim. Nutr. 94:130-137.

Ghazalah, A. A. and A. M. Ali. 2008. Rosemary Leaves as a Dietary Supplement for Growth in Broiler Chickens. Int. J. Poult. Sci. 7:234-239.

Huyghebaert, G., R. Ducatelle and F. V. Immerseel. 2011. An update on alternatives to antimicrobial growth promoters for broilers. The Veterinary Journal 187:182–188.

Jamroz, D., T. Wertelecki, M. Houszka and C. Kamel. 2006. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. J. Anim. Phys. Anim. Nutr. 90:255–268.

Jamroz, D., A. Wiliczkiewicz, T. Wertelecki, J. Orda and J. Skorupinska. 2005. Use of active substances of plant origin in chicken diets based on maize and locally cereals. Br. Poult. Sci. 46:485-493.

Jang, I. S., Y. H. Ko, S. Y. Kang and C. Y. Lee. 2007. Effect of commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed. Sci. Techn. 134:304–315.

Lan, Y., M. W. Verstegen, S. Tamminga and B. A. Williams. 2005. The role of the commensal gut microbial community in broiler chickens. World Poult. Sci. J. 61:95-104.

Lee, K. W., H. Everts., H. J. Kappert and A. C. Beynen. 2004. Growth performance of broiler chickens fed a carboxymethyl cellulose containing diet with supplemental carvacrol and/or cinnamaldehyde. Int. J. Poult. Sci. 3:619–622.

Lee, K. W., H. Everts and A. C. Beynen. 2004a. Essential oil in broiler nutrition. Poult. Sci. 3:738–752.

Lee, K. W., H. Everts, H. J. Kappert, M. Frehner, R. Losa and A. C. Beynen. 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br. Poult. Sci. 44:450-457.

Nasir, Z. and M. A. Grashorn. 2010. Effects of Echinacea purpurea and Nigella sativa supplementation on Broiler performance, carcass and meat quality. J. Anim. Feed Sci. 19:94–104.

Ocak, N., G. Erener, F. Burakak, M. Sungu, A. Altop and A. Ozmen. 2008. Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.) leaves as growth promoter source. Cz. J. Anim. Sci. 53:169–175.

Platel, K. and K. Srinivasan. 2000. Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. Nahrung (food) 44:42–46.

Steiner, T. 2009. Phytogenics in Animal Nutrition. Natural Concepts to Optimize Gut health and Performance. Nottingham University Press. Nottingham, United Kingdom.

Tekeli, A., L. Celik, H. R. Kutlu and M. Gorgulu. 2006. Effect of dietary supplemental plant extracts on performance, carcass characteristics, digestive system development, intestinal microflora and some blood parameters of broiler chicks. XII, EPC, 10-14 September, Verona, Italy.

Usha, N., S. Prakash and K. Srinivasan. 2010. Beneficial influence of dietary spices on the ultrastructure and fluidity of the intestinal brush border in rats. Br. J. Nutr. 104:31-39.

Williams, P. and R. Losa, 2001. The use of essential oils and their compounds in poultry nutrition. World Poult. 17:14–15.

Windisch, W. M., K. Schedle, C. Plitzner and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86:140-148.

Yasar, S. and J. M. Forbes. 1999. Performance and gastrointestinal response of broiler chicks fed on cereal grain-based foods soaked in water. Br. Poult. Sci. 40:65–76.