

EFFECTS OF ESSENTIAL OILS AND BENZOIC ACID ON BROILER PERFORMANCE AND GUT MICROFLORA

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Abstract

The concerns about possible antibiotic residues and disease resistance have given rise to great caution in the use of antibiotics in the animal industry and led to the removal of antimicrobial growth promoters from the diets. Among suitable natural alternatives, essential oils have gained considerable attention. Dietary acidification may be another alternative to antimicrobial growth promoters. Organic acids can positively affect performance possibly due to inhibition of intestinal bacteria competing with the host for available nutrients and due to reduction of toxic bacterial metabolites. In poultry production, organic acids are also used to sanitize the feed, considering problems with salmonella infections. Addition of essential oils and organic acids to poultry diets has been studied with varying and conflicting results.

The aim of the present study was to investigate the effect of an essential oil blend (EO) and benzoic acid on broiler performance, carcass characteristics and caecal microflora composition. One thousand male and female Cobb 500 chickens were used in total. There were eight replicates (four for males and four for females) for each one of the following five dietary treatments: T1 (basal diet with no added EO or benzoic acid), T2 (T1 with addition of 100 ppm EO), T3 (T1 with addition of 50 ppm EO and 250 ppm benzoic acid), T4 (T1 with addition of 25 ppm EO and 250 ppm benzoic acid) and T5 (T1 with addition of 50 ppm EO and 125 ppm benzoic acid). Chicken performance was measured at weekly intervals. At the end of week

four, eight chickens per treatment were sacrificed for determination of caecal microflora composition. At the end of the experiment (week six), 16 chickens per treatment were slaughtered, plucked and eviscerated for carcass yield determination. Dietary supplementation of each of the additives examined did not significantly affect chicken body weight, feed conversion ratio or mortality. Treatments did not significantly affect carcass or breast weight. The population of total aerobes, coliforms, total anaerobes, lactic acid bacteria, *Streptococcus* spp, *Clostridium* spp and *Clostridium perfringens* was unaffected by dietary treatments. Under the experimental conditions of this study, none of the examined feed additives nor their combinations had a significant beneficial effect compared to the unsupplemented controls. Essential oils as well as organic acids and their salts are known for their effect on gut microflora and have been used as alternatives to antibiotic growth promoters following a ban on their use in animal nutrition. It is highly probable that factors such as good hygiene, good health and optimal rearing of broilers, such as those in the present study, might have masked the potential effect of the dietary treatments.

**APPLYING HACCP SYSTEM AT AN ESTABLISHMENT PRODUCING
PELLETED COMPOUND FEEDS. II. OBSERVATIONS ON
PHYSICOCHEMICAL AND MICROBIOLOGICAL PARAMETERS OF
ANIMAL FEEDS**

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The present work constitutes a study to research information on data related to the hygiene of animal feeds. This investigation is based on the study of physicochemical and microbiological parameters of raw materials, premixes and compound feeds at different stages of the production process at an enterprise producing animal feeds, with a view to validating the food safety management system based on Hazard Analysis Critical Control Points (HACCP) principles. Three series of samples of 25 feeds (75 samples in total) were obtained in monthly intervals from a medium-scale feed manufacturer's enterprise. The samples consisted of five compound feeds in loose (at the mixer) or pelleted form, as well as 11 raw materials and four premixes used for their production. The analyses (825 in total) included measurements of water activity (α_w), pH, Total Bacterial Count (TBC), yeast and fungi, Enterobacteriaceae, coliforms, coagulase-positive staphylococci, *E. coli*, detection of *Salmonella* spp., *Listeria* spp. and determination of aflatoxin B₁. Water activity was maximum (0.686) for wheat bran and minimum for dried citrus pulp. The measurement of α_w ranged from 0.578 to 0.648 and 0.616 to 0.741, whereas pH ranged from 5.8 to 6.2 and 5.8 to 6.4 in loose and pelleted compound feeds, respectively. TBC in loose feeds ranged from 4.4 to 6.3; yeast –moulds 3.3 to 4.1, Enterobacteriaceae 3.2 to 4.7 and coliforms 3.2 to 4.9 log cfu g⁻¹. Microbiological counts of pelleted compound feeds were

remarkably lower for all the variables studied, compared to those of feeds in loose form. TBC in pelleted feeds ranged from <2.0 to $3.7 \log \text{ cfu g}^{-1}$. Wheat bran was the raw material with the highest microbial population. Coagulase-positive staphylococci were below the detection limit ($<2.0 \text{ cfu g}^{-1}$) and aflatoxin B₁ were not detected in any of the 75 samples and, furthermore, values for other variables were found to be negligible. *Escherichia coli* were detected in soybean meal, sunflower meal and in three out of 30 samples of compound feeds. *Listeria monocytogenes* and *Salmonella* spp. were not detected in any of the samples studied. The findings of the present work lead to the conclusion that the application of a food safety management system (HACCP) was implemented effectively in the feed enterprise, providing information that can be used for comparison purposes for the evaluation of systems in other similar feed manufactures. Moreover, with the recent adoption of Community legislation on Feed Hygiene, the information from the present study will contribute to appropriate measures adopted by the state to cope with EU laws on food and feed safety. Finally, the present information may be implemented for risk assessment by the European Food Safety Authority.

CRUDE PROTEIN AND AMINO ACID ILEAL DIGESTIBILITY OF FIELD PEA SEEDS (*PISUM SATIVUM* L.) IN BROILERS

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SUMMARY

An experiment was conducted to determine crude protein (CP) and amino acids (AAs) ileal digestibility of a local cultivar of field pea seeds (*Pisum sativum* L.) (FPS) in broiler chickens. Three experimental diets were used; a basal (B) and two others including FPS of the Greek cultivar “Olympos” at inclusion rates of either 150 or 300 g/kg, substituting equal amounts of maize starch. Hence, the differences in dietary CP and AAs concentrations resulted only from the inclusion of FPS. Chromium trioxide (Cr₂O₃) was included as an indigestible marker. Crude protein concentration in the complete diets ranged from 162 to 230 g/kg. All diets were fed *ad libitum* to broiler chickens between the age of 15 and 22 days. Six pens of 12 chickens each were randomly allocated into each treatment. Digesta were collected from all birds of each pen, from the section of the gastrointestinal tract between Meckel’s diverticulum and 2 cm anterior to the ileo-caeco-colonic junction. During the experimental seven-day period, mean feed intake of the basal diet was 53 g/bird/day, wherein it ranged from 57-62 g/bird/day and 61-67 g/bird/day for treatments with inclusion rates of FPS of 150 g/kg and 300 g/kg, respectively. Average body weight gain of the broilers was 173 g/bird for the basal diet and ranged from 282-335 g/bird and 337-364 g/bird for treatments containing 150 g/kg and 300 g/kg of FPS, respectively. The amounts of crude protein and AAs digested responded linearly to increased consumption while the relationships between consumed and digested amounts of AAs were determined by multiple linear regression using the slope for each one of them. Crude protein ileal digestibility of the diets showed a linear reduction by increasing inclusion rates of FPS from 0.87 for the basal diet to 0.81 and 0.78 for the diets containing 150 g/kg and 300 g/kg of FPS, respectively. A similar trend was noticed for the majority of AAs. The AAs of FPS with the highest values of ileal digestibility were, in decreasing order: arginine (0.92 ± 0.012), glutamic acid, lysine, methionine, phenylalanine and leucine, whereas those with the lowest values were cystine and valine (0.70 ± 0.022 and 0.72 ± 0.025, respectively). Ileal gross energy digestibility was similar between the basal diet and that which included FPS at the lower inclusion rate. To the contrary, the diet containing 300 g/kg of FPS had significantly (P<0.05) lower ileal digestibility than the other two (0.80 ± 0.014 and 0.78 ± 0.010 versus 0.072 ± 0.012 for the diets containing 0.150 or 300 g/kg of FPS, respectively). The multiple linear regression approach applied in this experiment was suitable for determining partial digestibilities in AAs of FPS without the need for consideration of basal endogenous AA secretions and losses.

Key words: *field pea seeds “Olympos”, broiler chickens, ileal digestibility, crude protein, amino acids, Cr₂O₃ marker, multiple linear regression approach.*

EFFECT OF NATURAL ZEOLITE (CLINOPTILOLITE) ON PERFORMANCE, CARCASS CHARACTERISTICS AND LITTER QUALITY OF BROILERS

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SUMMARY

The aim of this study was to assess the effect of clinoptilolite (a natural zeolite) on the performance and some carcass characteristics of broilers as well as on the quality of their litter. A total of 480 one-day-old Ross 308 broiler chickens were used in one comparative feeding trial. The experimental period lasted 42 days and consisted of three growth periods (1st: 1-14d, 2nd: 15-28d & 3rd: 29-42d). During this period, the broilers were given free and continuous access to three commercial diets (in mash form) which were either a typical diet (C) or three “zeolite diets” (Z₁, Z₂ and Z₃, which were supplemented with clinoptilolite at inclusion rates of 1, 2 and 3%, respectively). The broilers were randomly assigned to four experimental groups. Water was given *ad libitum*. The diets of the first period (1-14d) contained 22.2% CP & 3100 kcal of ME per kg of diet, while those of the second (15-28d) and third (29-42d) periods contained 21.5% CP & 3150 kcal of ME and 20.4% CP & 3200 kcal of ME, respectively. Diets were isocaloric and contained the proper levels of methionine, lysine, vitamins and minerals. Each group consisted of four replicates of thirty broilers each. Rice hulls were used as bedding. The average temperature was 24.5 ± 4.0 °C until the end of the experiment. Artificial lighting was set to provide 23h of light and 1h of darkness daily. Body weight gain and feed intake of all chickens from all pens were recorded weekly. At the 42nd day of age, eight chickens from each group weighing the average weight of pen were selected and slaughtered in order to determine dressing percentage, chemical composition of edible tissue, carcass colouring and leg quarter's length, weight and breaking strength. Excreta were collected for determination of moisture, phosphorus and calcium content, while litter was collected for moisture and total organic and nitrogen content determination. Average body weight gain was significantly (P<0.05) different between broilers of experimental groups. Broilers given diets Z₂ and Z₃ had a faster growth rate (P<0.05) and showed lower feed intake & better feed conversion ratio compared to those of the control group (C). The carcass colour became gradually lighter with the increase in dietary zeolite. Breaking strength of leg quarter was significantly (P<0.05) increased in the Z₁ & Z₂ groups and increased even more (P<0.01) in the group which contained the highest rate of zeolite (Z₃). Moisture and the content of calcium & phosphorus in the excreta were not affected. However, the addition of zeolite to broilers' diets resulted in a significant (P<0.05) decrease in the organic content of litter samples throughout the experimental period. Mean nitrogen concentration was not significantly affected by the addition of natural zeolite. The results of this study showed that the incorporation of clinoptilolite in the diet had a positive effect on broilers' performance, carcass colour of leg quarter and breast, breaking strength of leg quarter and, finally, on their litter quality.

Key words: broilers, clinoptilolite, performance, carcass traits, litter quality.

THE EFFECT OF NATURAL ZEOLITE ADDITION ON BROILER DIETS' DIGESTIBILITY

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SUMMARY

A digestibility experiment was conducted to estimate the effect of different levels of natural zeolite (clinoptilolite) addition on nitrogen balance, apparent digestibility of nutrients and apparent and true metabolizable energy of diets of broilers aged 28 days. The experiment lasted 15d (counting from the 28th d of age). The first four days were reserved for the adaptation of birds to cages, seven days was the duration of the preliminary period and four days were needed for the excreta collection. Fe₂O₃ (3 g/kg) was used as indigestible marker. A 2x4 factorial experimental design consisted of two sexes (male and female) and four feeding treatments (0, 1, 2 and 3% zeolite) were used. Forty-eight Ross broilers (24 for each sex) were placed in individual cages and randomly allocated into four groups (C, Z₁, Z₂, Z₃) so that each group consisted of six male and six female broilers. Daily feed intake was 80 g. Water was given *ad libitum*. The size of each individual cage was 0.20 m x 0.25 m x 0.20 m, thereby giving each bird about 0.05 m² of available space. The temperature was maintained at 24 ± 2 °C and artificial lighting was set to provide 16h of light and 8h of darkness daily. The broilers of group C (control) were fed a typical growing diet based on wheat, corn and soybean meal while the broilers of groups Z₁, Z₂ and Z₃ were fed the same diet to which 1, 2 and 3% of natural zeolite was added, respectively. Nitrogen of feed and excreta for each bird as well as metabolizable energy (ME) and crude protein (CP) of the experimental diets were determined. The procedure for the endogenous nitrogen losses estimation lasted 28h and was divided into four sub-periods as following: allowance of diet with marker for 4h, fasting period of 16h, allowance of N-free diet for 4h and finally re-allowance of diet with marker for another 4h. The composition of N-free diet was the following: sucrose (815 g/kg), corn seed oil (100 g/kg), ground wheat straw (30 g/kg) and vitamins & trace minerals premix (55 g/kg). Colourless excreta derived from N-free diet were used for the determination of endogenous nitrogen. Metabolizable energy of the experimental diets was estimated by using an adiabatic bomb calorimeter. Apparent and true metabolizable energy (AME & TME) of the diets were not significantly affected by the dietary supplementation of natural zeolite up to the level of 2% compared to the control diet. Also, the apparent digestibility of dry matter, organic matter and N-free extractives of the diet containing the highest inclusion rate of zeolite (3%) significantly (P<0.05) decreased as compared to the other three diets. The results of this study showed that the dietary supplementation of natural zeolite in broilers' diet at a level of up to 2% did not significantly affect the nitrogen balance nor the apparent and true metabolizable energy of experimental diets.

Key words: *natural zeolite, broilers, nutrients digestibility, apparent and true metabolizable energy.*

PERFORMANCE AND EGG QUALITY TRAITS OF LAYING HENS AS AFFECTED BY THE DIETARY SUPPLEMENTATION OF NATURAL ZEOLITE

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SUMMARY

A comparative feeding experiment was conducted to examine the effect of natural zeolite addition in the diet on the performance and egg quality traits of layers aged 28 weeks. The experiment lasted 56 days. Ninety-six Isa Brown hens of similar egg production capabilities and live weight were placed in wire cages in a naturally ventilated laying house and randomly allocated into four groups (C, Z₁, Z₂, Z₃), each group consisting of four replicates and each replicate of six hens, placed in two adjacent cages (three birds in each). Before the beginning of the experiment, the hens were reared together in standard housing, management and nutritional conditions. The layers of group C (control) were fed a typical egg production diet based on corn and soybean meal, while those of groups Z₁, Z₂ and Z₃ were fed the same diet to which was added 1, 2 and 3% of natural zeolite, respectively. The control diet (C) was calculated to contain 162 g/kg CP and 2750 kcal of ME/kg. The remaining diets contained 161, 159 and 157 g/kg CP as well as 2723, 2695 and 2668 kcal ME/kg for the treatments Z₁, Z₂ and Z₃, respectively. The temperature was maintained at 20.5 ± 2.5 °C and artificial lighting was set to provide 16h of light and 8h of darkness daily. Light 10 - 20 lux was provided from 07.00 to 23.00 h and darkness from 23.00 to 07.00. The hens were allowed *ad libitum* access to feed and water.

All birds were individually weighed at the beginning of the experimental period, at the middle and at 36 weeks of age. The feeds were weighed once weekly to determine feed consumption and feed conversion ratio. Egg yield was recorded daily for each cage. Total egg production, feed intake, feed conversion ratio and the changes in body weight of layers were measured. Also, the egg quality traits were determined. Total egg production, egg production rate, feed intake, feed conversion ratio as well as egg quality traits were not significantly affected by the dietary addition of natural zeolite to layers' diet. The layers of Z₂ and Z₃ groups showed a significant (P<0.05) decrease in their body weight gain compared to the control group (C) (183 g for the control group versus 111 and 136 g for the groups Z₂ and Z₃, respectively). The results of this study showed that the dietary supplementation of natural zeolite at an inclusion rate of up to 3% did not significantly affect the performance or the egg quality traits.

Key words: layers, natural zeolite, performance of layers, egg quality traits.