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## Volume 7 (2); June 25, 2017

## Research Paper

**Understanding the Direct and Indirect Mechanisms of Xylanase Action on Starch Digestion in Broilers.**

Lee SA, Wiseman J, O'Neill HVM, Scholey DV, Burton EJ and Hill SE.

*J. World Poult. Res.* 7(2): 35-47; pii:

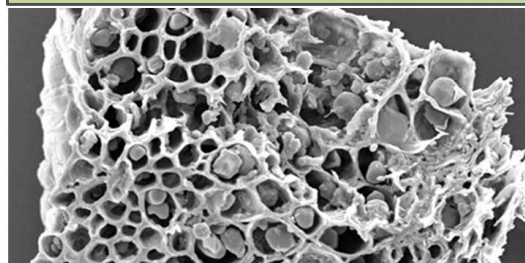
S2322455X1700006-7

**ABSTRACT:**

The objective of the current study was to investigate the mechanisms of xylanase action in a maize-soya diet and its effect on starch digestion. A total of 60 broilers were divided into 6 treatment groups; a control group without xylanase, and five other groups supplemented with xylanase (Econase XT 25; 100 g/t) from 1, 2, 3, 4 or 5 weeks before slaughter. At the end of the experiment, digesta was collected from the gizzard, upper and lower small intestine, and both caeca. Digesta pH ranged from pH 2.2-4.4, 5.9-6.6, 6.7-7.8 and 5.7-7.3 in the gizzard, upper small intestine, lower small intestine, and both caeca, respectively, with no effect of xylanase ( $P > 0.05$ ). Scanning Electron Microscope (SEM) images along with total starch measurements showed the progression of starch digestion through the tract. The SEM did not show any greater disruption to cell wall material with xylanase supplementation. This suggests that xylanase was not working directly on the cell wall and provides evidence for the hypothesis that xylanase works through an indirect mechanism. Peptide YY (PYY) concentration in the blood was higher during the first few weeks of supplementation, with longer periods of supplementation nulling this effect, implying that xylanase may be acting through a prebiotic mechanism. The RT-q PCR results revealed a numerical increase in glucose transporter (GLUT2 and SGLT1) expression at 2 and 3 weeks of xylanase supplementation, respectively, which might suggest a greater absorption capacity of birds. From these results, a potential mechanism of xylanase action in maize-based diets has been proposed.

**Key words:** Broiler, Maize, Starch, Xylanase

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**Xylanase in Broilers**

## Research Paper

**Effect of Blend Herbal Supplement on Haematology and Serum Biochemistry in Commercial Layer Chicken.**

Sakthi Priya M, Gopala Krishna Murthy TR and Vijayanand T.

*J. World Poult. Res.* 7(2): 48-56; pii:

S2322455X1700007-7

**ABSTRACT:**

Many herbal supplements are tested for their efficacy in poultry to replace the antibiotic growth promoter. Hence this study was carried out to find the effect of a blend herbal preparation (*Ocimum sanctum*, *Zingiber officinale*, *Allium sativum*, *Trigonella foenum graceum* and *Curcuma longa*) on the vital parameters in layers. An experimental trial for three weeks was carried out on 80 layers aged 11 weeks. Birds were randomly divided to 4 groups with 2 replicate for each group and supplemented by herbal preparation @ 0.1 percent, 0.25 percent and 0.5 percent in feed. The results indicated a significant increase in the red blood cells count, white blood cell count, total protein, albumin, globulin, calcium, phosphorus and reduction in the enzyme aspartate aminotransferase and alkaline phosphatase with alanine aminotransferase in the normal range. The significant increase in the vital parameters, decrease in hepatic enzymes inside the clinically healthy condition denote that the birds were in good health. Birds increased nutrient utilization, improved oxygen carrying capacity and caused active immune system. The better absorption of minerals like calcium and phosphorus signifies the role of herbs in enhancing digestion performance. Oral feeding caused a normal activity of hepatic enzymes which can prove safety and hepatoprotective nature of these herbs. Therefore, it can be concluded that supplementation of these herbs in the layer feed could be important in prevention of diseases in birds. However further studies are recommended to indicate the toxic levels of these herbs and optimize the beneficial dosage in diet of layer birds.

**Key words:** Enzymes, Haematology, Herbs, Layers, Serum biochemistry.

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**Herbal alternatives to antibiotics in Poultry**



## Research Paper

### Effects of Dietary Inclusion of Probiotics and Organic Acids on Performance, Intestinal Microbiology, Serum Biochemistry and Carcass Traits of Broiler Chickens.

Youssef IMI, Mostafa AS and Abdel-Wahab MA.

*J. World Poult. Res.* 7(2): 57-71; pii:

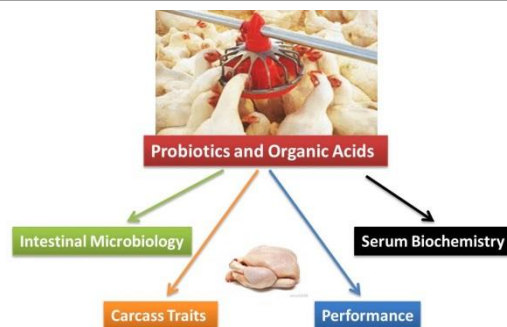
S2322455X1700008-7

#### ABSTRACT:

This study was conducted to evaluate the effects of probiotics and organic acids, as alternative feed additives to antibiotics, on productive performance of broilers. Two different types of probiotics varying in the microbial content were tested and organic acids blend was compared against a single organic acid (lactic acid). One hundred and ninety eight broiler chicks were randomly allocated into six treatments, each with 33 chicks. Every treatment consisted of 3 replicates with 11 birds per replicate. The dietary treatments were a control diet without any feed additives or the same control diet supplemented either with a commercial antibiotic (Maxus<sup>®</sup>G200), probiotics (Bactocell<sup>®</sup> or Biopellet-S<sup>®</sup>) or acidifiers (Salmo-Nil Dry<sup>®</sup> or lactic acid). The antibiotic was added to the diet at the rate of 0.005%, whereas the probiotics were used at 0.01%. The product Salmo-Nil Dry<sup>®</sup> was provided to the diet at a level of 0.4%, whereas the lactic acid was used at 0.20 %. It was found that the antibiotic, probiotics and lactic acid increased the body weight. All dietary supplements improved the FCR compared to the control. The additives reduced the serum cholesterol level and the pH of small intestine but did not affect the carcass yield, breast or organ weights. The feed supplements showed a numerical decrease in intestinal aerobes, fecal coliforms and *E. coli* counts. In addition, all additives significantly reduced total aerobic and staphylococcus counts in the carcass meat, with a numerical decrease in *E. coli* count. In conclusions, probiotics and acidifiers can be used as potential alternatives to antibiotics in broiler diets. No difference between the used types of probiotics was detected. Lactic acid alone seems to produce better performance results than the organic acid mixture. The effect of lactic acid produced by bacteria might be similar to that of the chemical one.

**Key words:** Broilers, Probiotic, Organic acids, Performance, Lactic acid, Carcass

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## Review

### Understanding of Social and Mating Behaviour of Ostrich (*Struthio camelus*).

Mukhtar N, Gazala and Waseem Mirza M.

*J. World Poult. Res.* 7(2): 72-78; pii:

S2322455X1700009-7

#### ABSTRACT:

The ostrich is the largest wild ratite bird. The head of ostrich is 1.8-2.75m above ground due to large legs. The ostrich is the largest vertebrate and achieves a speed of 60-65km/h. There are four extinct subspecies and limited to Africa. The preferred habitat in nature is the open area, small grass corners and open desert. They choose more open woodland and avoid areas of dense woodland and tall grass. In natural environment, ostrich is gregarious and lives in groups. This small crowd are led mature sire or dam. Walking, chasing and kantling are exhibited to protect the territories by males. Off springs are protected by adults from predator by mock injury. Other behaviours are yawning, stretching and thermoregulation. Frequency of mating is low in captivity. Mostly male-female ratio is 1:2 (Male: Female) kept in experiment and ostriches are selective in case of their mates and they might direct their courtship displays at humans rather than their mates, due to the presence of humans around in captivity. The breeding behaviour of ostriches is improved due to external application of L-carnitine-magnesium supplement.

**Keywords:** Ostrich, Mating, Behaviour, Courtship, Breeding

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## Research Paper

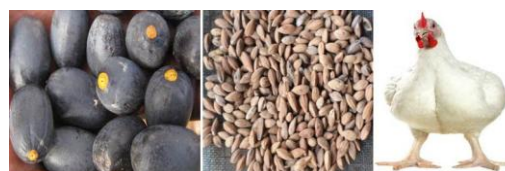
### Performances of Broiler Chickens Fed on Diet Supplemented with Thyme and Oregano Essential Oils Stabilized in a Plant Charcoal Matrix.

B Ngouana Tadjong R, Kana JR, Tsafack Necdem B, Yemdjie Mane DD, Mube Kuietche H, Kuiede S, Teguaia A and Meimandipour A.

*J. World Poult. Res.* 7(2): 79-87; pii: S2322455X1600010-7

#### ABSTRACT:

This study was designed to mitigate the volatile and oxidative ability of essential oils (EOs) in poultry feed using natural plant charcoal. The dietary treatments consisted of supplementing control diet (R<sub>0</sub>) with 0.01% of the mixture (1/1) of thyme and oregano EOs (R<sub>Th+Or</sub>), 0.2% of *Canarium* charcoal without EO (R<sub>0C</sub>), 0.2% charcoal respectively enriched with 0.01% of thyme EO (R<sub>0C+Th</sub>), oregano EO (R<sub>0C+Or</sub>) and the mixture of EOs (R<sub>0C+Th+Or</sub>). Results revealed a non-significant increase in weight gain for about 5 and 6%, respectively with the mixture of the EOs without charcoal and charcoal enriched with the mixture of the EOs compared to the control (R<sub>0</sub>). The carcass yield was higher with oregano EO and the mixture of EOs compared to the other treatments. Intestinal density was lower (P < 0.05) with the mixture of the EOs compared to thyme EO alone and the control ration. Charcoal containing EOs significantly increased (P < 0.05) total



protein in serum content, triglycerides, albumin, globulin and decreased serum content in creatinin, ASAT, ALAT and cholesterol. Hematological parameters were not significantly affected by the treatments. The blend of EOs associated or not to charcoal increased lactic acid bacteria count in both the ileum and the cæcum as compared to *E. coli* and salmonella. It was concluded that *Canarium* charcoal can be used to stabilize EOs in the feed for gut microbiota modulation and better growth performances of broiler chickens.

**Keywords:** Broiler, Essential oil, Hematology, Gut microbiota, Oregano, Plant charcoal, Thyme

[Full text-[PDF](#)] [XML] [DOAJ] [AGRIS]

## Research Paper

### Effect of Chemically Treated Litter on Ammonia Emission, Performance and Carcass Characteristics of Broiler Chicken.

Rashid A, Banday MT, Adil S, Khan AA, Qureshi S, Untoo M, and Pal MA.

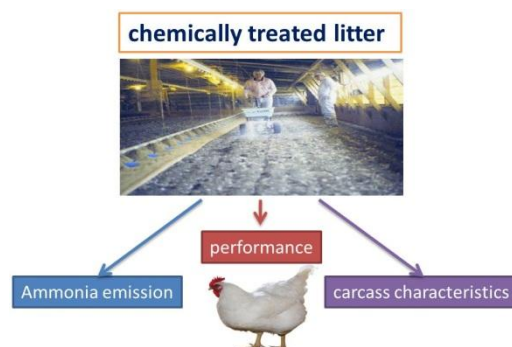
*J. World Poult. Res.* 7(2): 88-93; pii: S2322455X1700011-7

#### ABSTRACT:

The condition of litter is a single major factor in deciding the emission of various harmful gases particularly ammonia, which is a major environmental concern, affecting the overall welfare of birds. Therefore, a study was conducted with the objectives to assess the effect of two chemicals namely aluminum sulfate and calcium carbonate on litter ammonia emission, performance and carcass characteristics of broiler chicken. A total of 240 day old Cobb broiler chicks were randomly distributed into four treatment groups, each having 4 replicates of 13 chicks each. In the control group no chemical was added to litter; however, in other groups litter was treated with Aluminum Sulfate (AS) @ 25g/kg; Calcium Carbonate (CC) @ 50g/kg; and combination of 25g Aluminum Sulfate and 50g Calcium Carbonate/kg (ASCC). The results revealed a significant (P0.05) difference among themselves. AS was found to be highly effective in reducing the ammonia emission levels, either by itself or in combination, with values of  $9.46 \pm 0.35$  (AS) and  $10.499 \pm 0.39$  (ASCC) compared to  $47.7 \pm 2.40$  and  $51.15 \pm 1.85$  ppm in CC and control. A significant (P0.05) differences were found with respect to various carcass characteristics among treatment groups as compared to control. In conclusion, compared to CC, AS was found to be highly effective in reducing the litter ammonia emission and improving the performance of birds.

**Keywords:** Aluminium sulphate, Ammonia emission, Broiler chicken, Performance

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# Understanding the Direct and Indirect Mechanisms of Xylanase Action on Starch Digestion in Broilers

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## ABSTRACT

The objective of the current study was to investigate the mechanisms of xylanase action in a maize-soya diet and its effect on starch digestion. A total of 60 broilers were divided into 6 treatment groups; a control group without xylanase, and five other groups supplemented with xylanase (Econase XT 25; 100 g/t) from 1, 2, 3, 4 or 5 weeks before slaughter. At the end of the experiment, digesta was collected from the gizzard, upper and lower small intestine, and both caeca. Digesta pH ranged from pH 2.2-4.4, 5.9-6.6, 6.7-7.8 and 5.7-7.3 in the gizzard, upper small intestine, lower small intestine, and both caeca, respectively, with no effect of xylanase ( $P > 0.05$ ). Scanning Electron Microscope (SEM) images along with total starch measurements showed the progression of starch digestion through the tract. The SEM did not show any greater disruption to cell wall material with xylanase supplementation. This suggests that xylanase was not working directly on the cell wall and provides evidence for the hypothesis that xylanase works through an indirect mechanism. Peptide YY (PYY) concentration in the blood was higher during the first few weeks of supplementation, with longer periods of supplementation nulling this effect, implying that xylanase may be acting through a prebiotic mechanism. The RT-q PCR results revealed a numerical increase in glucose transporter (GLUT2 and SGLT1) expression at 2 and 3 weeks of xylanase supplementation, respectively, which might suggest a greater absorption capacity of birds. From these results, a potential mechanism of xylanase action in maize-based diets has been proposed.

**Key words:** Broiler, Maize, Starch, Xylanase

## INTRODUCTION

With an increasing demand from the biofuels industry for cereals such as maize to be used for ethanol production, the cost of such raw feed materials has risen in recent years. Consequently, considerable attention has been given to improve nutrient utilisation of these diets through the use of exogenous enzymes, including xylanases, to release more energy from the diet. Xylanase enzymes hydrolyse arabinoxylans, a major component of cereal grain cell walls (Collins et al., 2005). Traditionally, exogenous xylanases have been added to viscous diets,

such as those based on wheat, barley, and rye, whereby significant levels of soluble arabinoxylans have been demonstrated to have anti-nutritive effects on nutrient digestibility and absorption in poultry (Annison and Choct, 1991; Bedford and Schulze, 1998; Choct, 2006). These effects are often attributed to the ability of soluble non-starch polysaccharides (NSP) to increase digesta viscosity, thereby reducing digesta transit rate, digestibility, and increasing fermentation in the small intestine (Choct et al., 1996). Maize contains a relatively low concentration of soluble NSP (Choct, 1997) compared to other cereals, such as wheat, suggesting a possible



explanation for the lower magnitude of broiler response to xylanase supplementation in maize-based diets. However, there is evidence to suggest a beneficial effect of xylanase inclusion in maize-based diets through improved broiler performance (Zanella et al., 1999; Cowieson, 2010), indicating that additional mechanisms other than reduction in viscosity are important. It is important to note however, that these studies focus on the use of multi-enzyme applications and therefore the mechanisms of xylanase action exclusively must also be considered.

It is still unclear as to how such enzymes improve digestibility in maize-based diets. One well-discussed explanation is cell wall dissolution, whereby xylanases hydrolyse the insoluble NSP fraction in maize (Meng and Slominski, 2005). This presumably leads to the release of components, including starch, protein and lipids, from within the cell thereby positively aiding digestibility (Bedford, 2002). However, the site of enzyme action in the gut combined with digesta transit time suggests that there is insufficient time for exogenous xylanases to significantly degrade cell wall material directly by the small intestine. Therefore, it has been suggested that xylanases may be working via an indirect mechanism (Singh et al., 2012).

Endo-xylanase hydrolysis of arabinoxylans leads to the production of beneficial arabinoxyloligosaccharides (AXOS) (Broekaert et al., 2011). Courtin et al. (2008) reported a comparable improvement in Feed Conversion Ratio (FCR) of birds that were fed a maize diet supplemented with either xylanase or wheat bran oligosaccharides derived from xylanase-treated wheat bran. This suggested that oligosaccharides produced from xylanase degradation of fibrous material are influential to broiler performance. An increase in movement of xylo-oligomers to the caeca has been linked to an increase in volatile fatty acids (VFA) concentration through fermentation in this section (Choct et al., 1996, 1999). The presence of VFA in the intestinal lumen is suggested to promote peptide YY (PYY) release from endocrine L-cells, located predominantly in the distal ileum and colon, into the blood (Cuche et al., 2000). PYY is a neuropeptide that acts on the hypothalamus in the brain to delay gastric emptying, a process also known as ileal brake or gastroparesia (Pironi et al., 1993; Lin et al., 1996). Feed is therefore retained in the proventriculus and gizzard for longer, resulting in a finer 'grind' and improved protein digestion. As the protein coating the starch granules is digested, it allows for a greater access of the amylase to the granules, thereby improving starch digestibility.

It has previously been reported that PYY administration is capable of increasing the active uptake of

glucose in the small intestine (Bird et al., 1996; Croom et al., 1998). Glucose is transported across the enterocyte membrane via a sodium-glucose cotransporter (SGLT1). SGLT1 is a high affinity, low capacity transporter situated on the apical side of enterocytes (Braun and Sweazea, 2008). It actively transports glucose and galactose, along with two sodium ions down a concentration gradient from the intestinal lumen into the enterocyte (Kimmich and Randles, 1984). On the basolateral membrane of enterocytes, glucose transporter 2 (GLUT2) moves glucose out of the cell via facilitated diffusion. The bird's enhanced capacity for glucose absorption may be another possible explanation for the reported improvements in performance with xylanase supplementation, and therefore will be considered herein.

The current study was designed to investigate potential mechanisms by which xylanase may act in a maize-soya diet in order to elicit a beneficial response in broilers. The effects of xylanase on starch digestion were determined using both quantitative and qualitative methods to gain a more comprehensive understanding of the starch digestion process, and the factors influencing it.

## MATERIALS AND METHODS

### Ethical approval

The protocol for the experiment was reviewed and approved by Ethical Review Committee, University of Nottingham, and conducted according to the UK Home Office Animal (Scientific Procedures) Act of 2010.

### Animals, housing and diets

A total of 60 day-old male Ross 308 broiler chicks were supplied from a commercial hatchery and raised on a maize-soya diet (Target Feeds, UK; Table 1) with or without xylanase (Econase XT 25 at 100 g/t). Birds were group-housed in separate pens for a specific treatment group, with 10 birds per treatment. The limitation of replication in this study should be remembered when considering the data present herein. Treatment groups (6) included a control group fed a maize-soya diet without xylanase, and groups fed maize-soya diets supplemented with xylanase from 1, 2, 3, 4 or 5 weeks before slaughter. This xylanase preparation (Econase XT) contained 160,000 units of endo-1,4- $\beta$ -xylanase activity (EC 3.2.1.8) per gram. One xylanase unit (XU) is defined as the necessary amount of enzyme that liberates 1 nmol, reducing sugars from birch woodxylan, measured as xylose equivalents, under the conditions of the assay (AB Enzymes, Germany). The recovered xylanase activity in the diet was 18,100 BXU/kg, as measured using a

standardised ELISA courtesy of ESC (Enzyme Services and Consultancy). Chicks were kept in an appropriate warm environment with a progressive decrease in temperature from 35 to 21°C. Diets and water were provided *ad libitum* throughout the trial.

Five birds from each treatment group were euthanised on days 35 and 36 of age by an intravenous injection of pentobarbital, with cervical dislocation to confirm death. Digesta was collected by flushing contents from the gizzard, upper (to) and lower (from Meckel's Diverticulum) small intestine, and both caeca. Digesta pH was measured using an Inolab pH level 1 meter. The brachial vein under the wing was cut using a scalpel blade and blood glucose measured using an Accu-chek mobile blood glucose monitor (Roche, UK). Digesta samples were stored at -80 °C before being frozen in liquid nitrogen and freeze-dried. The empty weight of the gizzard and lengths of the upper and lower small intestine as well as both caeca were recorded. Approximately two sections of around 2 cm in length were removed from the proximal upper small intestine, snap frozen in liquid nitrogen, and stored at -80 °C, for later RNA extraction.

**Table 1.** Composition of raw materials and nutrient content of maize-soya diet

Ingredient	g/kg	Component	g/kg
Maize	624.5	AME MJ/kg	12.9
Soya extract hipro	260.0	Crude protein	204.3
Full fat soybean extruded	50.0	Calcium	9.4
L-lysine HCl	4.0	Available phosphorous	4.8
DL-methionine	4.0		
L-threonine	1.5		
Soya oil	20		
Limestone	12.5		
Monocalcium phosphate	15.0		
Sodium chloride	2.5		
Sodium bicarbonate	1.5		
Vitamin and Mineral premix	4.0		
Elancoban	0.5		

### Peptide YY analysis

Following cervical dislocation, blood was drawn from the aorta using a needle and syringe, and collected in a centrifuge tube at room temperature, to allow coagulation. Samples were then centrifuged at 1623 xg for 10 min to allow separation of serum. Serum was then stored at -20 °C, prior to using a chick specific PYY ELISA (Cusabio, China). Samples were analysed in duplicate using 25 µl serum, and repeated at 50 µl per well where samples were below detection limits. Sample PYY

concentrations were calculated using a specific plate reader program (Multiskan Ascent) calibrated with the kit standards, which were run for each plate assayed. Two quality control samples were also measured with each plate to monitor inter assay coefficient of variation. Samples with coefficients of variation greater than 15 % were repeated.

### Sample morphology

The morphology of starch granules from the digesta was examined using a Scanning Electron Microscope (SEM; Jeol JSM-6490LV) with the energy capacity of 15 kV. Samples were mounted onto aluminium stubs and gold coated using the sputter coater technique, before being viewed and photographed in the SEM unit.

### Starch fraction determination

A total starch assay (Megazyme International Ireland Ltd) was performed for the determination of starch, glucose and maltodextrins in digesta samples. Digesta samples were washed twice in 80 % ethanol and the precipitate digested with thermostable  $\alpha$ -amylase (300 U) and amyloglucosidase (20 U). Glucose release was measured using a glucose analyser (Analox GM9 Analyser). Both ethanol washes, assumed to contain soluble dextrin and glucose, were collected and the ethanol evaporated. Glucose concentration in this fraction was measured using the glucose analyser. Next, this soluble ethanol fraction was digested with  $\alpha$ -amylase (*A. oryzae*; 24 FAU; Sigma-Aldrich, UK; A8220) and amyloglucosidase (20 U), and glucose concentration measured again. The difference in glucose concentration before and after digestion gave the dextrin content.

### Glucose transporter gene expression

Total RNA was isolated from small intestine tissue samples using an RNeasy Fibrous Tissue Mini Kit (Qiagen Ltd, UK; 74704). Isolated RNA concentration and quality was then checked using a DS-11 spectrophotometer (DeNovix, USA). Purity of the RNA was verified by measurement of absorbance ratios at 260/280 and 260/230 nm, with ratios of approximately 2.0 indicating purity. Once RNA concentration for all samples was determined, 10 µl of sample was diluted with RNase-free water to give a final concentration of 100 ng/µl. Confirmation of this final concentration was achieved using the DS-11 spectrophotometer.

Synthesis of single-stranded complementary DNA (cDNA) from total RNA was performed using a Revert Aid First Strand cDNA Synthesis kit (Thermo Scientific, UK; K1622). Each reaction was performed in duplicate:

one containing the reverse transcriptase enzyme (+RT) and one where the enzyme was substituted with nuclease-free water (-RT). Another negative control was used in which total RNA was replaced with 3 µl nuclease-free water. This was intended to control for any genomic contamination in the RNA samples as well as other reaction components. Each reaction consisted of 3 µl total RNA, 1 µl random primer, 8 µl nuclease-free water, 4 µl 5X reaction buffer, 1 µl RiboLock RNase Inhibitor, 2 µl deoxynucleotide triphosphate (dNTP) mix and 1 µl RevertAid Reverse Transcriptase, for a total reaction volume of 20 µl. Reactions were run in a 96 well plate that was sealed with a film and placed into an Eppendorf Mastercycler Gradient thermal cycler. Conditions for reverse transcription were set for random hexamer primed synthesis, whereby samples were incubated at 25 °C for 5 min, followed by 42 °C for 60 min. The reaction was terminated by heating the samples to 70 °C for 5 min. The resulting cDNA products were stored at -20 °C.

Quantitative PCR of cDNA samples was performed using an Applied Biosystems StepOne Real-time PCR system with the primer sets listed in table 2. PCR plates (Applied Biosystems MicroAmp® Fast Optical 48-Well Reaction Plate, 4375816) were designed to assay 4 +RT samples, including a sample from treatment group 1 (control sample). Each reaction was run in triplicate for each gene. PCR was also run on the RT samples and a no template control (NTC) in which cDNA was replaced with nuclease-free water, to check for any contamination in the PCR components. The cDNA was diluted 1:3 with nuclease-free water to lower template concentration and to ensure there was enough sample for any repeats. A mastermix for each gene was made consisting of 200 µl GoTaq qPCR Mastermix (Promega UK Ltd; A6001), 4 µl gene specific forward primer, 4 µl gene specific reverse primer and 141.2 µl nuclease-free water. This mix was

then pulse centrifuged for 10 sec using a Thermo Scientific Espresso centrifuge. Into the appropriate wells, 18 µl of this mastermix and 2 µl cDNA were added. PCR was performed under the following conditions: 95 °C for 10 min and 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. A melt curve was produced at the end of the run to determine single product amplification. The comparative Ct method, also known as the 'delta-delta Ct' method, was used to quantify qPCR results. This method involved comparing the Ct values of the xylanase treated samples with the non-supplemented (control) samples. Both treated and control sample Ct values were normalised to the house-keeping gene.

$\Delta Ct = Ct \text{ value gene of interest} - Ct \text{ value control gene } (\beta\text{-actin})$

$\Delta\Delta Ct = \Delta Ct \text{ value gene of interest} - \text{Average } \Delta Ct \text{ value control sample}$

Fold change in gene expression was then calculated and averaged for the three replicates:

$$\text{Foldchange} = 2^{-\Delta\Delta Ct}$$

Agarose gel electrophoresis was used to confirm that qPCR amplified solely the gene of interest. PCR products were resolved on a 3 % agarose gel containing 5 % (v/v) ethidium bromide. One sample was selected from two different PCR plates that produced good melt curves. One replicate for each gene was selected and 4 µl 6X purple loading dye (New England Biolabs Inc, UK; B7024S) was added. Each dyed product (10 µl) was placed in a separate well on the gel. The products were separated, alongside a 20 bp low ladder (Sigma-Aldrich, UK; P1598), for approximately 1 h using 80 V. The gel was visualised and imaged under ultraviolet (UV) light using a transilluminator. The product band size was then determined by comparing positions relative to the ladder.

**Table 2.** Primer sequence used for qPCR analysis of glucose transporters

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product Size, bp
GLUT2	GTCCACCGCAAATGCTTCTAA	TGTCCCTGGAGGTGTTGGT	88
SGLT1	TTTGTATACAGATGTGGTGGC	GCATAAGCTCCACTACCATT	113
$\beta$ -actin*	GTCCACCGCAAATGCTTCTAA	TGCGCATTTATGGGTTTTGTT	78

\*Gilbert et al. (2008)

### Statistical analysis

Statistical significance was tested using the uni- and multi-variate platforms of IBM SPSS statistics v21 software. For statistically significant results ( $P < 0.05$ ),

Tukey's HSD Post-hoc test was performed to determine which groups in the population differ. For comparisons made between two sample groups, an independent sample t-test was performed.

## RESULTS

### Blood parameters

The effect of xylanase supplementation on blood glucose concentration of birds is shown in Figure 1. When comparing between treatment groups, no significant effect of supplementing diets with xylanase for any length of time was observed ( $P > 0.05$ ). However, when data was split between before and after 3 weeks of supplementation, a significant increase in glucose concentration was determined for birds fed diets supplemented with xylanase for 3 weeks or more ( $P < 0.05$ ).

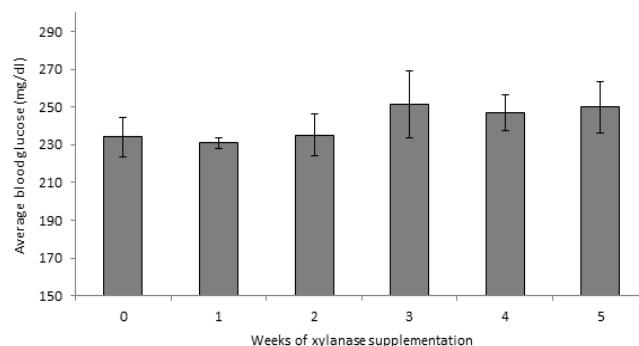
Changes in the circulating concentration of the gastrointestinal PYY hormone was also measured (Figure 2). When just the mean values are considered (Figure 2a), there appears to be an effect of supplementing diets with xylanase for a shorter period of time. Birds supplemented for 3 weeks showed a significantly higher PYY concentration in the blood compared to the non-supplemented control birds ( $P < 0.05$ ). Feeding xylanase over a longer time period appears to reverse this effect. However, when considering the raw data (Figure 2b), no significant effect was seen due to the large data variance ( $P > 0.05$ ,  $R = 0.0931$ ).

### Changes in gastrointestinal tract

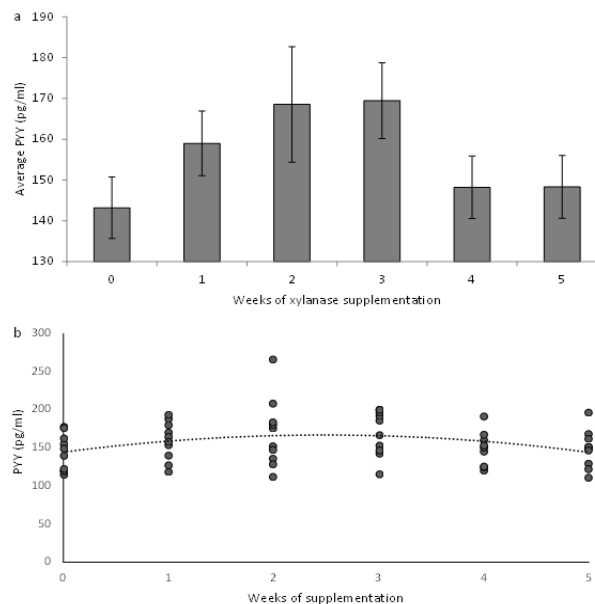
For all treatment groups, digesta pH ranged from pH 2.2-4.4 in the gizzard, 5.9-6.6 in the upper small intestine, 6.7-7.8 in the lower small intestine and 5.7-7.3 in both caeca. For each traction section, digesta pH did not significantly differ with xylanase treatment for any length of time ( $P > 0.05$ ).

Gizzard weight as well as intestine and caeca length were measured (Table 3) to assess the potential effect of xylanase supplementation on the physiology of digestive tract organs. The length of the upper small intestine and both caeca were not significantly affected by treatment ( $P > 0.05$ ). However, the length of the lower small intestine significantly increased in birds fed a xylanase supplemented diet for 3 weeks before slaughter compared to the non-supplemented control birds ( $P > 0.05$ ). Although birds supplemented with xylanase for 3 weeks had the highest mean gizzard weight, overall gizzard weight was not significantly affected by treatment ( $P > 0.05$ ).

An increase in digesta weight collected from the caeca of birds supplemented with xylanase for longer periods of time was shown (Table 4). When data was split between before and after 3 weeks of xylanase supplementation there was a significant increase in caecal digesta weight in birds fed xylanase diets for 3 or more weeks ( $P < 0.05$ ).



**Figure 1.** Average blood glucose concentration of broilers supplemented with xylanase for 0-5 weeks before slaughter. Error bars denote  $\pm$  standard error of the mean (from 10 bird replicates).



**Figure 2.** Peptide YY hormone concentration of broilers supplemented with xylanase for 0-5 weeks before slaughter. Graphs show averaged data (a) and raw data (b) sets. Error bars denote  $\pm$  standard error of the mean (from 10 bird replicates, except week 4 of supplementation which has 9 replicates).



**Table 3.** Effect of length of xylanase supplementation on gizzard weight, and intestinal and caecal length of broilers at 5 weeks of age

Weeks of supplementation	Weight (g)				Length (mm)			
	Gizzard	SEM	USI	SEM	LSI	SEM	Caeca	SEM
0	42	1.49	632	20.78	669 <sup>b</sup>	14.02	154	3.91
1	43	0.89	711	21.66	727 <sup>b</sup>	30.34	159	4.40
2	41	1.68	663	15.00	686 <sup>b</sup>	18.03	160	4.90
3	45	1.12	691	15.25	754 <sup>a</sup>	16.12	158	3.77
4	42	1.69	637	18.81	713 <sup>b</sup>	23.09	155	5.32
5	43	2.00	669	21.02	709 <sup>b</sup>	13.99	158	5.58

SEM = Standard Error of the Mean (from 10 bird replicates); USI- upper small intestine; LSI- lower small intestine; <sup>ab</sup>Mean values not sharing a common superscript letter are significantly different at  $P < 0.05$

**Table 4.** Effect of length of xylanase supplementation on the weight of digesta collected from each tract section of broilers at 5 weeks of age

Weeks of supplementation	Digesta weight (g DM)							
	Gizzard	SEM	USI	SEM	LSI	SEM	Caeca	SEM
0	6.35	0.68	3.08	0.28	3.21	0.22	0.31	0.03
1	6.28	0.45	3.12	0.39	2.74	0.34	0.30	0.03
2	6.78	0.27	3.18	0.22	2.66	0.36	0.27	0.03
3	6.56	0.30	3.50	0.33	3.17	0.22	0.34	0.05
4	5.74	0.50	2.63	0.34	2.48	0.21	0.34	0.04
5	6.57	0.55	3.14	0.33	3.16	0.19	0.41	0.07

SEM = Standard Error of the Mean (from 10 bird replicates); DM- dry matter; USI- upper small intestine; LSI- lower small intestine

### Starch digestion

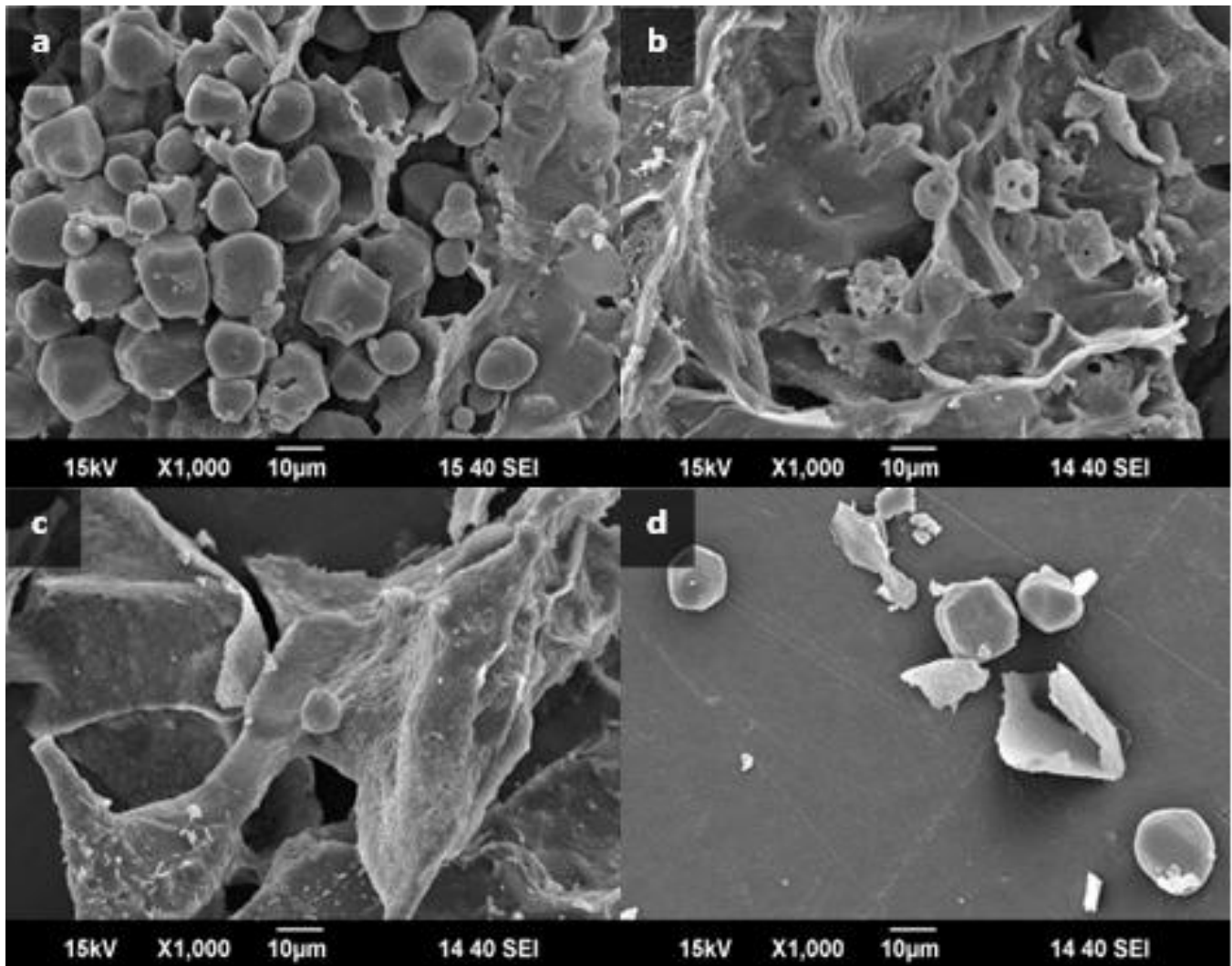
Total starch results demonstrated the progression of starch granule digestion as digesta passes through the digestive tract of birds (Figure 3). The most noticeable starch content was found in the gizzard (Figure 3a), with decreased levels within the upper small intestine and then less again in the digesta of the lower small intestine ( $P < 0.05$ ) as starch becomes hydrolysed by endogenous amylases. Dextrin content varied with tract section and treatment with no consistent trend (Figure 3b). As expected, the glucose content (Figure 3c) of gizzard digesta was minimal, while a higher ( $P < 0.05$ ) glucose content was found in the upper small intestine, as this is the major site of starch digestion. By the lower small intestine, glucose levels fall again as monomers are absorbed by enterocytes. The content of starch, dextrin and glucose in any tract section was not significantly affected by treatment ( $P > 0.05$ ).

Total starch results were also supported by SEM imaging (Figure 4) where starch granules appear to become more hydrolysed and are fewer in number as digesta enters more distal tract sections. Interestingly, a small number of starch granules escaped digestion and

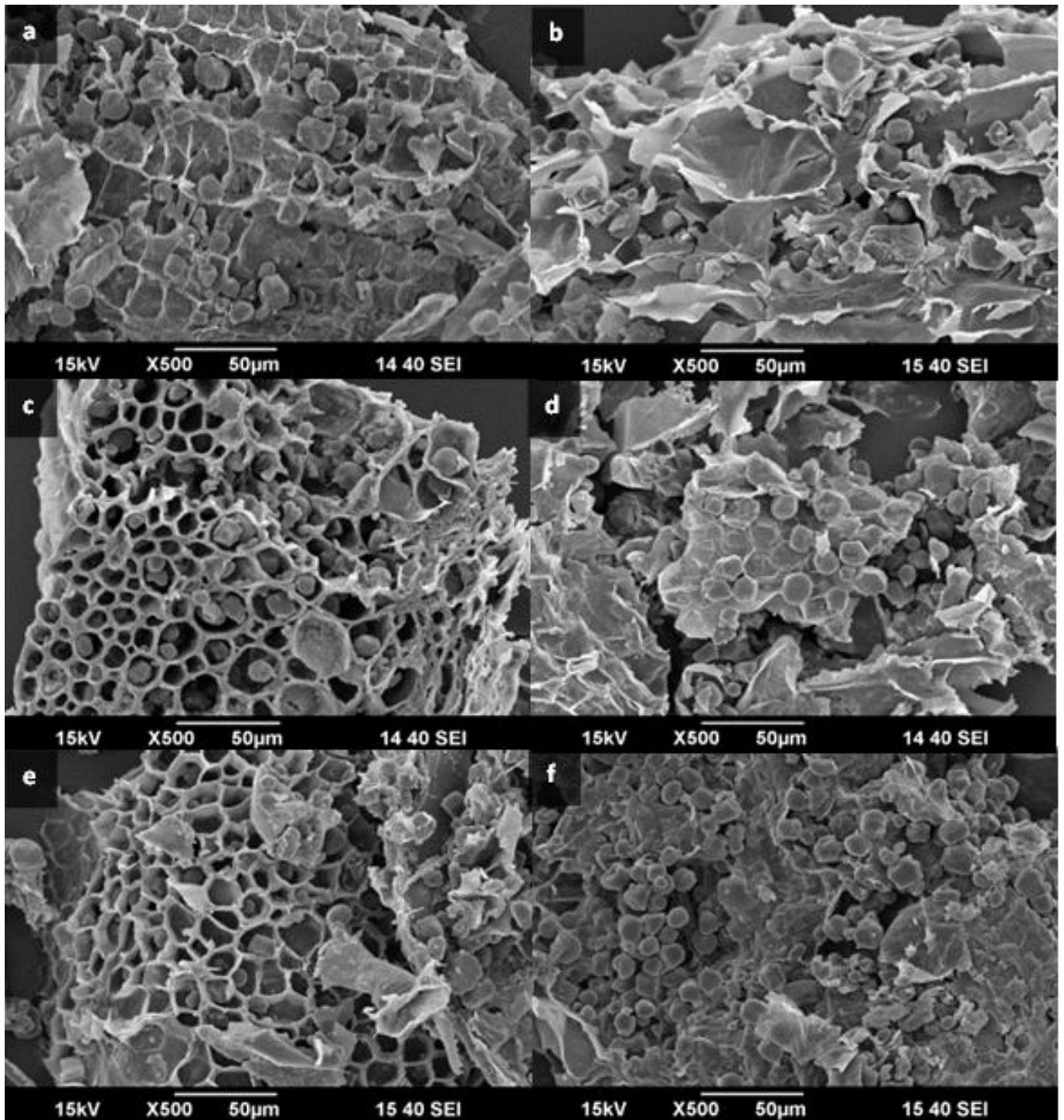
entered the caeca (Figure 4d) for all treatment groups. All gizzard samples showed cell wall fractionation (Figure 5), however greater apparent cell wall destruction with xylanase treatment was not evident.

### Glucose transporter expression in the small intestine

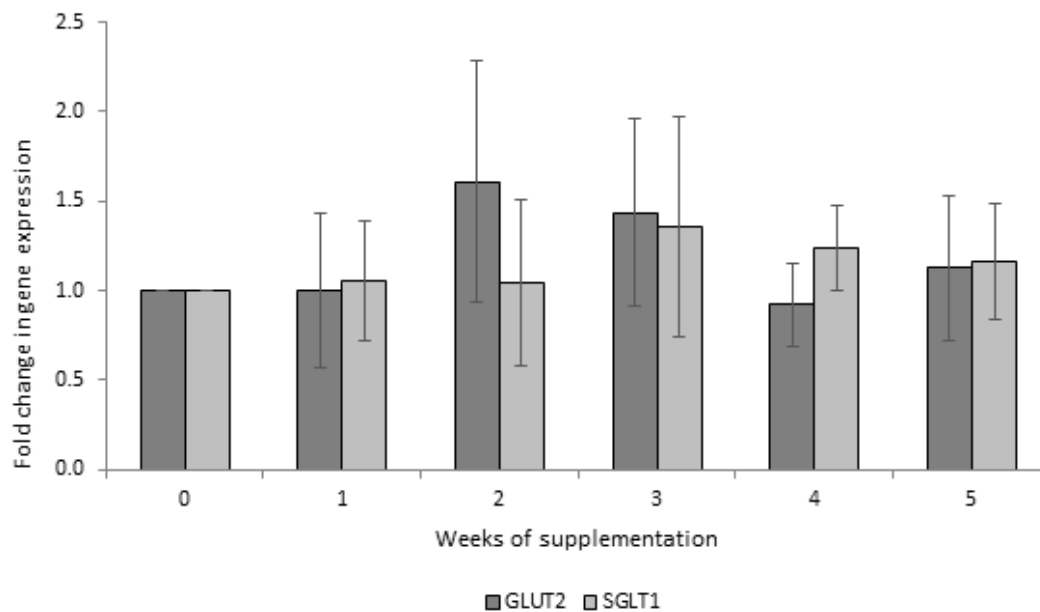
Fold change in expression of the glucose transporter genes, GLUT2 and SGLT1, are shown in Figure 6. Gel electrophoresis confirmed the GLUT2, SGLT1 and  $\beta$ -actin product sizes of 88 bp, 113 bp and 78 bp, respectively (Figure 7). Due to the large variation in qPCR results, GLUT2 and SGLT1 gene expression was not shown to vary significantly with length of xylanase supplementation ( $P > 0.05$ ). However, results do show a numerical increase in GLUT2 expression at 2 weeks of xylanase supplementation. GLUT2 expression reduced and plateaued as the length of xylanase supplementation increased. A similar trend was observed for SGLT1 expression. However the initial increase in expression started slightly later, at 3 weeks of xylanase supplementation.



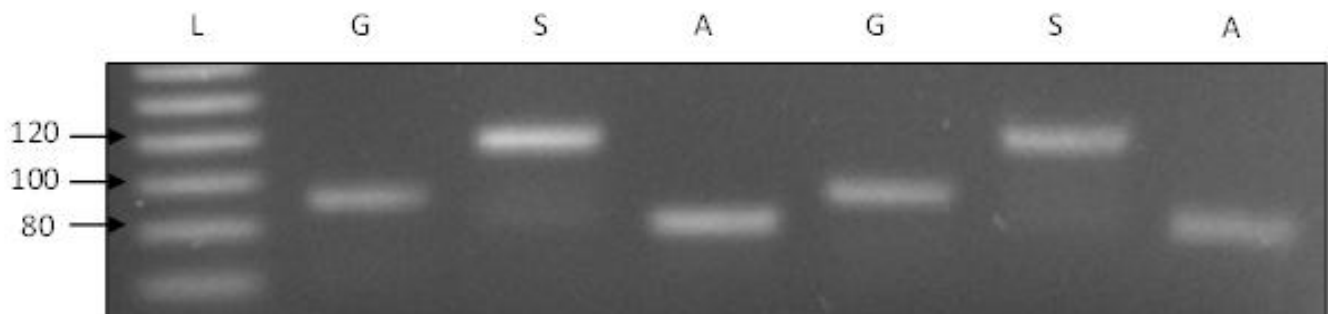
**Figure 4.** SEM images showing the progression of starch granule digestion through the tract of broilers at 5 weeks of age. Starch granules in digesta taken from the gizzard (a), upper small intestine (b), lower small intestine (c) and caeca (d).



**Figure 5.** Starch granules in gizzard digesta taken from broilers supplemented with xylanase for 0-5 weeks before slaughter. SEM images show control broilers (a) and broilers supplemented with xylanase 1 (b), 2 (c), 3 (d), 4 (e) and 5 (f) weeks before slaughter.



**Figure 6.** Expression of GLUT2 and SGLT1 gene in the small intestine of broilers supplemented with xylanase for 1-5 weeks before slaughter. Data are presented as a fold change in gene expression relative to control (non-supplemented) bird gene expression. Error bars denote  $\pm$  standard error of the mean (from 10 bird replicates, except weeks 2 and 3 of supplementation which have 9 replicates).



**Figure 7.** Agarose gel separation of qPCR reaction products. Products for the GLUT2 (G), SGLT1 (S) and  $\beta$ -actin (A) genes were run against a 20 bp ladder (L) for determination of product length.

## DISCUSSION

NSP may play an important role in the physical entrapment of starches and proteins in cereal, thereby limiting their digestibility (Aftab, 2012). Disruption of the cell wall matrix through xylanase action is expected to release these entrapped components making them more available to endogenous enzymes (Bedford, 1996). This concept is supported by reports of improved starch, protein and fat digestibility in maize-soya diets when supplemented with exogenous xylanase containing enzyme cocktails (Zanella et al., 1999; Meng and Slominski, 2005; Cowieson et al., 2010). However, SEM

images revealed no apparent difference in the matrices surrounding the starch granules for diets with or without xylanase supplementation, although the subjective nature of the SEM should be taken into account. Moreover, since birds were fed *ad libitum* prior to slaughter, feed particles at various stages of digestion would be expected to be present along the tract. Xylanase activity of 18,100 BXU/kg in feed was confirmed in the current study and with reports of fungal xylanase activity in digesta (Vahjen and Simon, 1999), although this was not measured in the current work, it can be assumed that the xylanase was also active in the tract. In contrast to the reported release of starch granules from their cell wall matrices with addition



of xylanase to maize *in vitro* (Masey O'Neill et al., 2012), the mechanism of xylanase action *in vivo* may not be as apparent due to the highly complex nature of the digestive tract. Moreover, the reasoning may not be solely dependent on plant cell wall material, but may also contain protein matrices that can form a continuous network that encapsulates starch granules, thereby preventing their release. Recently, however, Gonzalez-Ortiz et al. (2017) reported changes to the structural integrity of wheat cell walls in response to xylanase, using an auto-fluorescent technique. This method allows arabinoxylan structure to be specifically visualised (Jääskeläinen et al., 2013), unlike standard microscopy, and thus might explain the lack of findings when using the latter technique.

Total starch measurements also revealed no significant effect of xylanase on starch digestion through the tract. However, it was evident that starch still remained in the lower small intestine with some granules entering the caeca. Weurding et al. (2001) reported the same total tract starch digestion values as ileal starch digestion, indicating that the undigested starch fraction was not fermented in the caeca. Carre (2004) suggested that while soluble molecules or free granules can enter the caeca, as shown in the present study, the majority are expected to bypass the caeca. Therefore, caecal fermentation of starches may not be significant for the completion of starch digestion.

In addition to a direct action on cell wall material, xylanase may also play an indirect role on diet digestibility. Singh et al. (2012) reported increased secretion of PYY with xylanase supplementation to a maize-soya diet. In addition, Neyrinck et al. (2012) also described an increase in blood plasma PYY concentration with AXOS inclusion into the diet of mice, suggesting that it is the products of NSP hydrolysis that are affecting gastrointestinal hormone secretion. Caecal fermentation of AXOS produced from arabinoxylan breakdown by xylanase may promote PYY secretion leading to increased feed retention in the proventriculus and gizzard. This results in a 'finer grind' of feed material, with potential for greater gizzard development and improved protein digestion in the gastric phase. Since starch granules in the endosperm of the maize kernel are held within cellular and proteinaceous matrices, disruption of these materials can lead to granule release and enhancement of starch digestibility. In the current study, PYY secretion increased significantly when birds were fed maize-soya diets supplemented with xylanase for 1-3 weeks. This might suggest that the response is greater when birds are supplemented with the enzyme for a shorter period of time compared to birds supplemented over the whole trial

period, indicating an adaptive effect due to the age of birds when supplementation begins. When birds were exposed to the enzyme at a young age they are able to adapt to these changes, most likely through modification of the microflora population. Enhanced fermentation in the small intestine and caeca, due to changes in the microbial population, leads to greater production of VFA and thus lactic acid in these sections, resulting in a reduced pH (Gao et al., 2008). Xylanase supplementation has been shown to reduce fermentation in the small intestine, while increasing caecal fermentation and VFA production (Choct et al., 1999). As no change in intestinal or caecal pH was observed in the current study, this might suggest that there had not been any change in gut microflora due to xylanase supplementation. However, laboratory conditions may have not allowed for a considerable change in microflora. Therefore, this could be the reason for limited enzyme response, particularly during short supplementation periods, although further microbial analysis would be needed to confirm these results.

Greater feed retention in the gizzard due to the ileal brake mechanism has been linked to increased gizzard weight (Masey O'Neill et al., 2014). However, in the current work, empty gizzard weight was unaffected by xylanase supplementation. This may also be due to the physical form of the diet, as mash diets, such as the feed in this study, have already been shown to increase gizzard weight when compared to pelleted diets (Niret al., 1995). Therefore, the change in gizzard weight may not be as noticeable when feeding a mash diet, when the gizzard is already well developed. Moreover, intestinal length was not shown to be significantly affected by xylanase addition. Xylanase supplementation at 1000 XU/kg diet has been shown to reduce the relative weight and length of the small intestine (Wuet al., 2004) in broilers fed a wheat-based diet for 21 days. As viscosity is less of a concern in a maize-based diet, this may be the reason why there is not a consistent change in intestinal length with enzyme addition.

Previously, PYY administration has also been shown to increase active uptake of glucose in the small intestine of mice (Bird et al., 1996). This was achieved without a significant change in energy expenditure, suggesting that PYY may also enhance the efficiency of glucose absorption in the small intestine. Interestingly, other reports have shown that PYY administration *in ovo* can improve growth and FCR of chicks during the first week post-hatch (Coles et al., 1999; 2001), an effect attributed to an enhanced absorptive capacity. Croom et al. (1999) postulated that *in ovo* PYY administration may increase glucose transporter maturation thereby enhancing glucose

absorption during post-hatch growth when digestive and absorptive processes are not yet fully developed. Glucose transporter response to PYY in older birds has not yet received much attention. As xylanase supplementation has been shown to increase PYY secretion in birds, there is consequently a potential for an enhanced absorptive capacity in these birds.

Glucose is absorbed from the intestinal lumen into epithelial cells by SGLT1, and then leaves at the basolateral membrane by GLUT2, before entering blood circulation. Guo et al. (2014) reported a decrease in SGLT1 expression in broilers fed a wheat-based diet when supplemented with xylanase. In contrast, in the current study, increased expression of the glucose transporters, GLUT2 and SGLT, although non-significant, may suggest a potential response to xylanase inclusion in maize-based diets. Miyamoto et al. (1993) reported an increase in SGLT1 and GLUT2 expression in the jejunum of rats fed a high-glucose diet compared to those fed a low-carbohydrate diet. This would suggest that a higher concentration of glucose in the intestine causes a response that elevates glucose transporter expression. As diet digestibility has been found to improve with xylanase supplementation of wheat and maize-based diets (Choct et al., 1999; Cowieson, 2005; Choct, 2006), this could increase glucose content of intestinal digesta. However in the current work, the glucose content of digesta taken from the upper small intestine was not significantly affected when birds were fed xylanase. However, if the absorptive capacity of birds increased with increasing glucose concentration then differences in luminal glucose content may be difficult to detect. When considering the concentration of absorbed glucose, studies have reported no effect of xylanase on blood glucose concentration in broilers (Gao et al., 2007; Luo et al., 2009). However, in the current study, blood glucose concentration increased in birds fed xylanase for 3 or more weeks. This is in agreement with Singh et al. (2012) who reported an increase in serum glucose concentration of broilers fed a xylanase supplemented maize-soya diet for 42 d. Xylanase supplementation is assumed to improve nutrient digestion and absorption in the small intestine of broilers (Choct et al., 1999). Therefore, the rise in blood glucose level after 3 weeks of feeding xylanase could be due to greater starch digestion and glucose absorption. Although it should also be noted that the number of bird replicates per treatment was relatively small (n = 10), from the aforementioned trends in treatment response a potential mechanism of xylanase action can be proposed. Xylanase induced secretion of PYY may enhance starch digestion, due to the aforementioned 'ileal-brake mechanism', thereby

increasing glucose concentration in the intestinal lumen. Subsequent promotion of glucose transporter expression could increase glucose absorption and thus provide more energy to the bird for growth. With further investigation, this may give a potential mechanism for the improvement in performance seen in birds fed maize-based diets supplemented with xylanase.

## CONCLUSION

In the current study, the progression of starch granule digestion was followed through the digestive tract, giving an indication as to where and how starches are being digested. Xylanase supplementation did not appear to have any significant effect on starch digestion in broilers nor on cell wall degradation. However, a potential indirect mechanism of xylanase action has been proposed that may explain the reported improvements in performance when birds are fed maize-based diets supplemented with xylanase.

### Consent to publish

Not applicable

### Competing interests

The authors declare that they have no competing interests

### Authors' contributions

The present study was funded by AB Vista, Marlborough, Wiltshire, UK. Sophie Lee, Julian Wiseman, Helen Masey O'Neill and Sandra Hill contributed to the conception, design and interpretation of data. Sophie Lee was also involved in the collection of data, statistical analysis and drafting of the manuscript. Dawn Scholey and Emily Burton executed PYY analysis by ELISA at their facilities at Nottingham Trent University. All authors read and approved the final manuscript.

## REFERENCES

- Aftab U (2012). Exogenous carbohydrase in corn-soy diets for broilers . *World's Poultry Science Journal*, 68: 447-463.  
DOI:<https://doi.org/10.1017/S0043933912000566>
- Annison Gand choct M (1991). Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. *World's Poultry Science Journal*, 47: 232-242.  
DOI:<https://doi.org/10.1079/WPS19910019>
- Bedford MR (2002). The role of carbohydrases in feedstuff digestion. *Poultry Feedstuffs: Supply*,

- Composition and Nutritive Value, 26: 319-336. DOI: [10.1079/9780851994642.0319](https://doi.org/10.1079/9780851994642.0319)
- Bedford MR and schulze H (1998). Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews*, 11: 91-114. DOI: 10.1079/NRR19980007
- Bedford MR (1996). The effect of enzymes on digestion. *Journal of Applied Poultry Research*, 5: 370-378. DOI: <https://doi.org/10.1093/japr/5.4.370>
- Bird AR, Croom WJ JR, Fan YK, Black BL, McBride BW and Taylor IL (1996). Peptide regulation of intestinal glucose absorption. *Journal of Animal Science*, 74: 2523-2540. DOI: 10.2527/1996.74102523x
- Broekaert WF, Courtin CM, Verbeke K, Van de Wiele T, Verstraete W and Delcour JA (2011). Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Critical Reviews in Food Science and Nutrition*, 51: 178-194. DOI: 10.1080/10408390903044768
- Carre B (2004). Causes for variation in digestibility of starch among feedstuffs. *World Poultry Science*, 60: 76-89. DOI: <https://doi.org/10.1079/WPS20036>
- Choct M (1997). Feed non-starch polysaccharides: chemical structures and nutritional significance. *Feed Milling International*, 7: 13-26. DOI: <https://doi.org/10.1071/AN15276>
- Choct M (2006). Enzymes for the feed industry; past, present and future. *World's Poultry Science Journal*, 62: 5-16. DOI: <https://doi.org/10.1079/WPS200480>
- Choct M, Hughes RJ and Bedford MR (1999). Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. *British Poultry Science*, 40: 419-422. DOI: 10.1080/00071669987548
- Choct M, Hughes RJ, Wang J, Bedford MR, Morgan AJ and Annison G (1996). Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science*, 37: 609-621. DOI: <http://dx.doi.org/10.1080/00071669608417891>
- Coles BA, Croom J, Daniel LR, Christensen VL and Eisen EJ (2001). In ovo peptide YY administration improves body weight at hatch and day 3 in turkey poults. *The Journal of Applied Poultry Research*, 10: 380-384. DOI: <https://doi.org/10.1093/japr/10.4.380>
- Coles BA, Croom WJ, Brake J, Daniel LR, Christensen VL, Phelps CP, Gore A and Taylor IL (1999). In ovo peptide YY administration improves growth and feed conversion ratios in week-old broiler chicks. *Poultry Science*, 78: 1320-1322. DOI: <https://doi.org/10.1093/ps/78.9.1320>
- Collins T, Gerday C, Feller G (2005). Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiology Reviews*, 29: 3-23. DOI: 10.1016/j.femsre.2004.06.005
- Courtin CM, Broekaert WF, Swennen K, Lescroart O, Onagbesan O, Buyse J, Decuyper E, Van de Wiele T, Marzorati M and Verstraete W (2008). Dietary inclusion of wheat bran arabinoxyloligosaccharides induces beneficial nutritional effects in chickens. *Cereal Chemistry*, 85: 607-613. DOI: <http://dx.doi.org/10.1094/CCHEM-85-5-0607>
- Cowieson AJ (2005). Factors that affect the nutritional value of maize for broilers. *Animal Feed Science and Technology*, 119: 293-305. DOI: <https://doi.org/10.1016/j.anifeedsci.2004.12.017>
- Cowieson AJ (2010). Strategic selection of exogenous enzymes for corn/soy-based poultry diets. *Journal of Poultry Science*, 47: 1-7. DOI: 10.2141/jpsa.009045
- Cowieson AJ, Bedford MR and Ravindran V (2010). Interactions between xylanase and glucanase in maize-soy-based diets for broilers. *British Poultry Science*, 51: 246-257. DOI: 10.1080/00071661003789347
- Croom WJ, McBride B, Bird AR, Fan YK, Odle J, Froetschel M and Taylor IL (1998). Regulation of intestinal glucose absorption: A new issue in animal science. *Canadian Journal of Animal Science*, 78: 1-13. DOI: 10.4141/A97-056
- Croom WJ, Brake J, COLES BA, Havenstein GB, McBride BW, Peebles ED and Taylor IL (1999). Is intestinal absorption capacity rate-limiting for performance in poultry. *Journal of Applied Poultry Research*, 8: 242-252. DOI: <https://doi.org/10.1093/japr/8.2.242>
- Cuche G, Cuber JC and Malbert CH (2000). Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 279 : G925-G930.
- Gao F, Jiang Y, Zhou GH and Han ZK (2008). The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut microflora in broilers fed on wheat-based diets. *Animal Feed Science and Technology*, 142: 173-184. DOI: <https://doi.org/10.1016/j.anifeedsci.2007.07.008>
- Gao F, Jiang Y, Zhou GH and Han ZK (2007). The effects of xylanase supplementation on growth, digestion, circulating hormone and metabolite levels, immunity and gut microflora in cockerels fed on wheat-based diets. *British Poultry Science*, 48: 480-488. DOI: <http://dx.doi.org/10.1080/00071660701477320>
- Gilbert ER, Emmerson DA, Webb KEJ and Wong E (2008). Dietary protein quality and feed restriction influence abundance of nutrient transporter mRNA in the small intestine of broiler chicks. *Journal of Nutrition*, 138: 262-271.

- Gonzalez-Ortiz G, Sola-Oriol D, Martinez-Mora M, Perez JF, Bedford MR (2017). Response of broiler chickens fed wheat-based diets to xylanase Supplementation. *Poultry Science*, [In Press]. DOI: 10.3382/ps/pex092
- Guo S, Liu D, Zhao X, Li C and Guo Y (2014). Xylanase supplementation of a wheat-based diet improved nutrient digestion and mRNA expression of intestinal nutrient transporters in broiler chickens infected with *Clostridium perfringens*. *Poultry Science*, 93: 94-103. DOI: 10.3382/ps.2013-03188
- Jääskeläinen A-S, Holopainen-Mantila U, Tamminen T and Vuorinen T (2013). Endosperm and aleurone cell structure in barley and wheat as studied by optical and Raman microscopy. *Journal of Cereal Science*, 57:543–550. DOI:https://doi.org/10.1016/j.jcs.2013.02.007
- Lin HC, Zhao XT, Wang L and Wong H (1996). Fat-induced ileal brake in the dog depends on peptide YY. *Gastroenterology*, 110: 1491-1495. DOI: http://dx.doi.org/10.1053/gast.1996.v110.pm8613054
- Luo D, Yang F, Yang X, Yao J, Shi Band Zhou Z (2009). Effects of xylanase on performance, blood parameters, intestinal morphology, microflora and digestive enzyme activities of broilers fed wheat-based diets. *Asian-Australasian Journal of Animal Sciences*, 22: 1288-1295. DOI: https://doi.org/10.5713/ajas.2009.90052
- Masey O'Neill HV, Singh M and Cowieson AJ (2014). Effects of exogenous xylanase on performance, nutrient digestibility, volatile fatty acid production and digestive tract thermal profiles of broilers fed on wheat- or maize-based diet. *British Poultry Science*, 55: 351-359. DOI: 10.1080/00071668.2014.898836
- Masey O'Neill HV, Lui N, Wang JP, Diallo A and Hill S (2012). Effect of xylanase on performance and apparent metabolisable energy in starter broilers fed diets containing one maize variety harvested in different regions of China. *Asian-Australasian Journal of Animal Science*, 25: 515-523. DOI: 10.5713/ajas.2011.11314
- Meng X and Slominski BA (2005). Nutritive values of corn, soybean meal, canola meal and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. *Poultry Science*, 84: 1242-1251. DOI: https://doi.org/10.1093/ps/84.8.1242
- Miyamoto K, Hase K, Takagi T, Fujii T, Taketani Y, Minami H, Oka T and Nakabou Y (1993). Differential responses of intestinal glucose transporter mRNA transcripts to levels of dietary sugars. *Biochemical Journal*, 295: 211. DOI: https://doi.org/10.1042/bj2950211
- Neyrinck AM, Van Hée VF, Piront N, De Backer F, Toussaint O, Cani PD and Delzenne NM (2012). Wheat-derived arabinoxylan oligosaccharides with prebiotic effect increase satietogenic gut peptides and reduce metabolic endotoxemia in diet-induced obese mice. *Nutrition and Diabetes*, 2: E28. DOI: 10.1038/nutd.2011.24
- Nir I, Hillel R, Ptichi I and Shefet G (1995). Effect of particle size on performance. 3. Grinding pelleting interactions. *Poultry Science*, 74: 771-783. DOI: https://doi.org/10.3382/ps.0740771
- Pironi L, Stanghellini V, Miglioli M, Corinaldesi R, De Giorgio R, Ruggeri E, Tosetti G, Poggioli G, Morselli Labate AM, Monetti N, Gozetti G, Barbara L, and Go VLW (1993). Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma level of peptide YY. *Gastroenterology*, 105: 733-739. DOI: https://doi.org/10.1016/0016-5085(93)90890-O
- Singh A, Masey O'Neill HV, Ghosh TK, Bedford MR and Haldar S (2012). Effects of xylanase supplementation on performance, total volatile fatty acids and selected bacterial populations in caeca, metabolic indices and peptide YY concentrations in serum of broiler chickens fed energy restricted maize-soybean based diets. *Animal Feed Science and Technology*, 177: 194-203. DOI: https://doi.org/10.1016/j.anifeedsci.2012.08.005
- Vahjen W and Simon O (1999). Biochemical characteristics of non starch polysaccharide hydrolyzing enzyme preparations designed as feed additives for poultry and piglet nutrition. *Archives of Animal Nutrition*, 52: 1-14. DOI: http://dx.doi.org/10.1080/17450399909386147
- Weurding RE, Veldman A, Veen WA, vander Aar PJ and Verstegen MW (2001). Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. *Journal of Nutrition*, 131: 2329-2335.
- Wu YB, Ravindran V, Thomas DG, Birtles MJ and Hendriks WH (2004). Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *British Poultry Science*, 45: 76-84. DOI: http://dx.doi.org/10.1080/00071660410001668897
- Zanella I, Sakomura NK, Silversides FG, Figueirdo A and Pack M (1999). Effect of enzyme supplementation of broiler diets based on corn and soybeans. *Poultry Science*, 78: 561-568. DOI: https://doi.org/10.1093/ps/78.4.561





## Effect of Blend Herbal Supplement on Haematology and Serum Biochemistry in Commercial Layer Chicken

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### ABSTRACT

Many herbal supplements are tested for their efficacy in poultry to replace the antibiotic growth promoter. Hence this study was carried out to find the effect of a blend herbal preparation (*Ocimum sanctum*, *Zingiber officinale*, *Allium sativum*, *Trigonella foenum graecum* and *Curcuma longa*) on the vital parameters in layers. An experimental trial for three weeks was carried out on 80 layers aged 11 weeks. Birds were randomly divided to 4 groups with 2 replicate for each group and supplemented by herbal preparation @ 0.1 percent, 0.25 percent and 0.5 percent in feed. The results indicated a significant increase in the red blood cells count, white blood cell count, total protein, albumin, globulin, calcium, phosphorus and reduction in the enzyme aspartate aminotransferase and alkaline phosphatase with alanine aminotransferase in the normal range. The significant increase in the vital parameters, decrease in hepatic enzymes inside the clinically healthy condition denote that the birds were in good health. Birds increased nutrient utilization, improved oxygen carrying capacity and caused active immune system. The better absorption of minerals like calcium and phosphorus signifies the role of herbs in enhancing digestion performance. Oral feeding caused a normal activity of hepatic enzymes which can prove safety and hepatoprotective nature of these herbs. Therefore, it can be concluded that supplementation of these herbs in the layer feed could be important in prevention of diseases in birds. However further studies are recommended to indicate the toxic levels of these herbs and optimize the beneficial dosage in diet of layer birds.

**Key words:** Enzymes, Haematology, Herbs, Layers, Serum biochemistry.

### INTRODUCTION

Antibiotic growth promoters in livestock and poultry production are practiced for many years to promote the growth and to improve the feeding efficiency thereby improving the health of the animal and the birds. But the inclusion of these feed additives increases not only the cost of production but also increases the development of resistant microbes and produces residues in meat and eggs (Sojoudi et al., 2012; Yang et al., 2009). In the present scenario, the use of in - feed- antibiotics is under threat after the ban of the antibiotic use by the European Union since January 2006. This ban has given the increasing prevalence of resistance to antibiotics in chicken (Kabir, 2009) and escalated the search for alternatives for use in

the poultry industry (Janardhana et al., 2009). Also the recent changes in legislation on the use of antimicrobials, altered feed requirements and more efficient birds necessitate the need for an alternative treatment. Hence many International Institutions and organizations related to public health are showing deep concern to reduce the use of antibiotics in poultry (Gatne et al., 2010). World Health Organization (WHO) has started to give emphasis on the development and use of herbal products for the benefit of world population in viewing the limitations and ill effects of chemical drugs (Jinsuklee, 2004).

Currently natural alternatives like probiotics, prebiotics, plant extracts and the essential oils are gaining importance as alternative supplement (Pirogozliev et al.,

2008; Yang et al., 2008; Ayasan, 2013). The important advantages claimed of medicinal plants in various ailments are their safety besides being economical, clinically effective and their easy availability. Medicinal plants compete with synthetic drugs and most of them do not have any residual effects (Tipu et al., 2006). Many herbal plant preparations are prescribed to strengthen host resistance (Thatte and Dahanakur, 1989) due to their immunomodulatory activities. Immunity breakdown will make the harmful bacteria to take upper hand as the gastro intestinal tract microflora is a mixture of fungi, protozoa and bacteria comprising both beneficial and harmful microorganisms (Gabriel et al., 2006). Harmful bacteria may be involved in localized or systemic infections, intestinal putrefaction, and toxin formation (Jeurissen et al., 2002). Hence, to get rid of the mortality associated with harmful luminal pathogens viz., *Salmonella*, *Escherichia coli* (*E.coli*), *Clostridium* sp. and other pathogenic bacteria and to improve the gut health, modulate the immune response and to aid in digestion and absorption herbal extracts can be used as an alternative treatment.

Cardelle et al. (2010) stated that the *Allium sativum* (garlic) exerts a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria. Bharkava et al. (2012) reported that *Zingiber* (Zinger) contain potent antimicrobial property against *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*. Ashish et al. (2013) investigated the methanol and aqueous extract of *Ocimum sanctum* (tulsi) for antimicrobial activity on *E.coli*, *Proteus mirabilis* and *Staphylococcus aureus* and found that *E.coli* and *Proteus mirabilis* were resistant to both the extracts, with *Staphylococcus aureus* sensitive to both the extracts. *Trigonella foenum-graecum* (fenugreek) seeds have been reported to possess immunomodulatory, antibacterial, anti-inflammatory, antioxidant, antinociceptive, gastroprotective and anxiolytic properties (Virupaksha et al., 2007). Basu et al. (2009) proved that the fenugreek was found to strongly inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in *in vitro* studies. Deshmukh (2014) investigated the effect of *Curcuma longa* (turmeric) against enteric pathogens. The study showed that *E.coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella typhimurium* were very much sensitive to turmeric extract as compared to other tested pathogens. He also concluded that the turmeric possesses curcumin, which is responsible for antibacterial activity of turmeric.

Hence the present study was taken to assess the effect of *Ocimum sanctum*, *Allium sativum*, *Curcuma longa*, *Trigonella foenum-graecum* and *Zingiber officinale* on haematology and serum biochemistry on supplementation in feed as several reports are available indicating the role of these herbs in immunomodulatory, growth promoting, antibacterial, anti-cancerous, anti oxidant, anti-inflammatory, hepatoprotective and antioxidant properties (Reuter et al., 1996; Rivlin, 2001; Vijayalakshmi et al., 2011; Shukla, et al., 2012).

## MATERIALS AND METHODS

The present study was carried out at a private layer farm at Pudhur, Vaiyappamali, Thiruchengodu taluk of Namakkal district, Tamil Nadu, India. A total of 80 Lohman breed layers aged 11 weeks and in the weight range of  $751.67 \pm 16.07$ g were used for present study. The birds were allotted at random into four experimental groups with two replicates (10 birds per replicate), viz., Control group (Regular feed without any herbal preparation) as mentioned in table 1, Group I (Regular feed with 0.1% herbal preparation), Group II (Regular feed with 0.25% herbal preparation) and Group III (Regular feed with 0.5% herbal preparation). The trial lasted for 21 days (11-14 weeks). Proper management, necessary vaccinations (before the initiation of the trial) and good environmental conditions were maintained throughout the study period. The herbs *Ocimum sanctum* (Tulsi), *Allium sativum* (Garlic), *Zingiber officinale* (ginger), *Trigonella foenum-graecum* (Fenugreek) and *Curcuma longa* (curcumin) were purchased fresh from market. Their rinds (ginger and garlic) were peeled off using knife, washed, shade dried and later ground to smooth powder and stored separately in an air tight container. 100 g of each herb was taken, blend and packed in an air tight plastic container for further use.

### Haematological parameters

Blood samples were collected from wing vein of the chicken using sterile needles and syringes from both the control and the treated groups (six birds from each group) before initiation and 21 days after the initiation of the experimental trial. The blood samples were collected into well labelled and sterilized bottles containing Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant. The samples were investigated for the following haematological parameters; Total Erythrocyte Count (TEC), Haemoglobin content (Hb), Packed Cell Volume (PCV) and White Blood Cell count (WBC) as stated in the method of Campbell (1998).

### Biochemical parameters of blood

Blood samples were collected from the wing vein of birds from both the control and the treated groups (six birds from each group) before initiation and 21 days after the initiation of the experimental trial. Serum was separated and stored at -20°C until analysis. The samples were investigated for the following biochemical parameters; Total proteins, albumin, globulin, calcium, phosphorus, potassium and enzymes like Aspartate Amino Transferase (AST), Alanine amino Transferase (ALT) and Alkaline Phosphatase (ALP) in UV-VIS double beam spectrophotometer (Systronics, Model 2202, India)

### Ethical approval

Approval was given by the local advisory committee members of the Post Graduate Diploma in Ethno Veterinary practices course of Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

### Statistical analysis

The least mean square was analysed on the data collected on various parameters and the significant difference was estimated by the Duncan's Multiple Range test using SPSS 20.0 software. P values less than 0.05 were reported as statistically significant.

## RESULTS AND DISCUSSION

The present work involving the supplementation of herbal preparation to assess its effect on haematology and serum biochemistry in layer chicken at 11-14 weeks of age (growers) was carried out in field conditions to look for the alternative phytobiotic growth promoting substances in improving health and production, without any issues of drug - residues and antimicrobial resistance.

### Haematological parameters

The effect of the herbal preparations on haematological parameters is presented in the table 2 and figure1.

The mean Hb (gram %) is significantly ( $P < 0.05$ ) increased in all the treatment groups including the control group (after initiation) than the control group (before initiation). Hence the treatment does not have any influence on Hb level in the bird's body. The mean RBC ( $\times 10^6/\text{cu.}\mu\text{l}$ ) and the mean WBC ( $\times 10^6/\text{cu.}\mu\text{l}$ ) count was also found to be significantly ( $P < 0.05$ ) increased in the treatment groups suggesting that the herbal preparation is found to stimulate RBC production at a slightly increased level of supplementation. Similarly PCV (%) was

significantly increased in the groups II and III than the control groups.

There is no significant difference ( $P < 0.05$ ) in heterophils (%) value, lymphocytes (%) value, monocytes (%) value, eosinophil (%) value and basophil (%) value between the control groups and the treatment groups.

### Blood biochemistry

The effect of the herbal preparations on the blood biochemistry is presented in the table 3 and figure 2.

The total protein content (g/dl), albumin content and the globulin content of the treatment groups were significantly increased when compared with the control groups (before and after initiation). However globulin content in is not significantly higher than the control.

The glucose content in Group I is significantly ( $P < 0.05$ ) reduced and Group III is significantly ( $P < 0.05$ ) increased than the control group. The calcium (mg) in treatment groups was comparatively higher than the control groups. The phosphorus (mg) in Group I was significantly ( $P < 0.05$ ) higher than the control groups. There is no significant ( $P < 0.05$ ) difference in potassium (m.equ) between the treatment groups and the control groups. The enzyme ALT (U/L) is significantly ( $P < 0.05$ ) increased in group I and group III than the other groups. The enzymes AST (U/L) and ALP (U/L) is significantly ( $P < 0.05$ ) reduced in the treatment groups than the control groups.

**Table 1.** Regular feed composition for layers (11-14 weeks)

Ingredients	Quantity (Kg)
Maize	600
Pellet SF(36%) protein	97
Soya bean meal	150
Deoiled rice bran	115
Calcite	15
Dicalcium Phosphate	12
Salt	4
Soda bicarbonate	1
DL methionine	1
Lysine	0.9
Choline chloride	1
Vitamins	500 gram
Traceminerals	1 kg
Phytase	100 gram
Toxin binder	1 kg
Liver powder	500 gram

Protein = 17.25%; Energy = 2700Kcal

**Table 2.** Effect of the herbal preparations on the haematological parameters in layer chicken aged 11 -14 weeks (n=6)

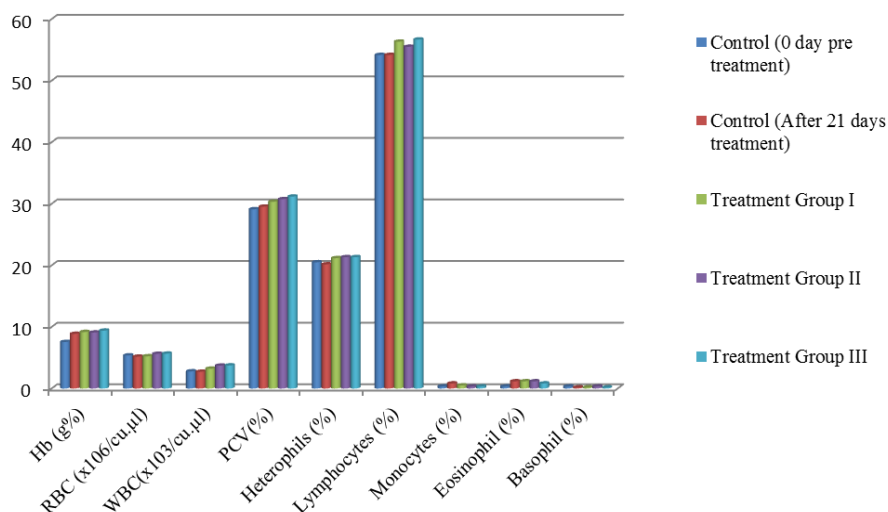
Variables	Treatment (Mean±SE)				
	Control (Days)		Group I	Group II	Group III
	Pretreatment at day 0 (samples from all the groups)	After 21 days treatment period (Control group)			
Hb (g%)	7.55 ± 0.29 <sup>a*</sup>	8.88 ± 0.19 <sup>b</sup>	9.18 ± 0.83 <sup>b</sup>	9.10 ± 0.03 <sup>b</sup>	9.4 ± 0.05 <sup>b</sup>
RBC (x10 <sup>6</sup> /cu.µl)	5.37 ± 0.09 <sup>a</sup>	5.17 ± 0.05 <sup>a</sup>	5.23 ± 0.03 <sup>a</sup>	5.63 ± 0.09 <sup>b</sup>	5.66 ± 0.12 <sup>b</sup>
WBC (x10 <sup>3</sup> /cu.µl)	2.78 ± 0.15 <sup>a</sup>	2.72 ± 0.25 <sup>a</sup>	3.22 ± 0.11 <sup>b</sup>	3.72 ± 0.14 <sup>c</sup>	3.75 ± 0.11 <sup>c</sup>
PCV (%)	29.11 ± 0.80 <sup>ab</sup>	29.53 ± 0.72 <sup>a</sup>	30.33 ± 0.80 <sup>ab</sup>	30.75 ± 0.51 <sup>c</sup>	31.17 ± 0.49 <sup>c</sup>
Heterophils (%)	20.5 ± 0.43 <sup>a</sup>	20.17 ± 0.48 <sup>a</sup>	21.17 ± 0.60 <sup>a</sup>	21.33 ± 0.95 <sup>a</sup>	21.33 ± 0.62 <sup>a</sup>
Lymphocytes (%)	54.16 ± 0.60 <sup>a</sup>	54.17 ± 0.60 <sup>a</sup>	56.33 ± 1.23 <sup>a</sup>	55.50 ± 1.65 <sup>a</sup>	56.67 ± 1.12 <sup>a</sup>
Monocytes (%)	0.33 ± 0.21 <sup>a</sup>	0.83 ± 0.31 <sup>b</sup>	0.50 ± 0.31 <sup>b</sup>	0.33 ± 0.17 <sup>b</sup>	0.33 ± 0.17 <sup>ab</sup>
Eosinophil (%)	0.33 ± 0.21 <sup>a</sup>	1.17 ± 0.17 <sup>a</sup>	1.17 ± 0.22 <sup>a</sup>	1.17 ± 0.21 <sup>a</sup>	0.83 ± 0.21 <sup>a</sup>
Basophil (%)	0.33 ± 0.21 <sup>a</sup>	0.17 ± 0.17 <sup>a</sup>	0.17 ± 0.17 <sup>a</sup>	0.33 ± 0.21 <sup>a</sup>	0.17 ± 0.17 <sup>a</sup>

\*Mean values within the same row with different superscripts differ significantly (P<0.05)

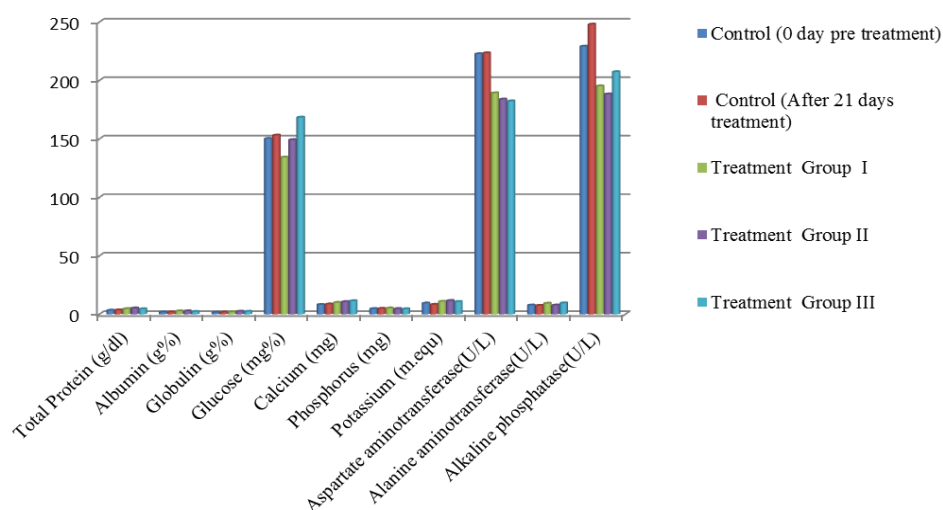
**Table 3.** Effect of the herbal preparations on the blood biochemical parameters in layer chicken aged 11 -14 weeks (n=6)

Variables	Treatment (Mean±SE)				
	Control (Days)		Group I	Group II	Group III
	Pretreatment at day 0 (samples from all the groups)	After 21 days treatment period (Control group)			
Total Protein (g/dl)	3.31 ± 0.17 <sup>*</sup>	3.58 ± 0.03 <sup>a</sup>	4.69 ± 0.02 <sup>b</sup>	5.21 ± 0.03 <sup>c</sup>	4.56 ± 0.11 <sup>b</sup>
Albumin (g%)	1.75 ± 0.07 <sup>a</sup>	1.84 ± 0.08 <sup>a</sup>	2.71 ± 0.01 <sup>b</sup>	2.75 ± 0.01 <sup>b</sup>	2.23 ± 0.01 <sup>c</sup>
Globulin (g%)	1.56 ± 0.22 <sup>a</sup>	1.74 ± 0.09 <sup>ab</sup>	1.97 ± 0.02 <sup>b</sup>	2.46 ± 0.03 <sup>c</sup>	2.33 ± 0.11 <sup>c</sup>
Glucose (mg%)	150.28 ± 0.87 <sup>a</sup>	153.12 ± 0.39 <sup>b</sup>	134.26 ± 0.10 <sup>c</sup>	149.23 ± 0.19 <sup>a</sup>	168.41 ± 0.18 <sup>d</sup>
Calcium (mg)	8.21 ± 0.10 <sup>a</sup>	8.64 ± 0.03 <sup>b</sup>	9.98 ± 0.03 <sup>c</sup>	10.67 ± 0.01 <sup>d</sup>	11.39 ± 0.08 <sup>e</sup>
Phosphorus (mg)	4.66 ± 0.12 <sup>ab</sup>	4.88 ± 0.06 <sup>b</sup>	5.18 ± 0.03 <sup>c</sup>	4.69 ± 0.13 <sup>ab</sup>	4.56 ± 0.04 <sup>a</sup>
Potassium (m.equ)	9.44 ± 0.12 <sup>ab</sup>	8.31 ± 1.59 <sup>a</sup>	10.90 ± 0.01 <sup>b</sup>	11.60 ± 0.21 <sup>b</sup>	10.72 ± 0.11 <sup>b</sup>
Aspartate aminotransferase (U/L)	222.72 ± 1.14 <sup>a</sup>	223.49 ± 0.14 <sup>a</sup>	189.24 ± 0.33 <sup>b</sup>	183.91 ± 0.30 <sup>c</sup>	182.33 ± 0.35 <sup>c</sup>
Alanine aminotransferase (U/L)	7.68 ± 0.11 <sup>a</sup>	7.46 ± 0.12 <sup>a</sup>	9.26 ± 0.06 <sup>b</sup>	7.73 ± 0.05 <sup>a</sup>	9.51 ± 0.16 <sup>b</sup>
Alkaline phosphatase (U/L)	229.17 ± 7.83 <sup>a</sup>	248.03 ± 1.24 <sup>b</sup>	195.22 ± 1.11 <sup>c</sup>	188.31 ± 0.53 <sup>c</sup>	207.34 ± 1.70 <sup>d</sup>

\*Mean values within the same row with different superscripts differ significantly (P<0.05)

**Figure 1.** Effect of the herbal preparations on the haematological parameters in layer chicken aged 11 -14 weeks





**Figure 2.** Effect of the herbal preparations on the blood biochemical parameters in layer chicken aged 11 -14 weeks

Our findings determined significant increase in RBC, white blood cells and PCV which are comparable with the findings of Vivian et al. (2015), Ajit et al. (2012) and Mitruka and Rawnsley (1977) who opined that the increase in the vital hematological constituents like PCV, Hb, RBC, and WBC in birds fed with the herbal ingredients ginger, garlic and tulsi is an indication of improved oxygen carrying capacity of the cells which translated to a better availability of nutrients for utilization to the birds consequently affecting their well being with an active immune system. Gole (2001) reported an increase in total leukocyte count in broilers supplemented with 1% *Ocimum sanctum* and liquid preparation of vitamin E and Se. The non significant increase of haemoglobin with the value falling within the normal range is in accordance with the results of Onu et al. (2010) who observed similar results on feeding ginger and garlic to broiler and with the findings of Kolte (1993) who reported significant increase in Hb% in the broilers fed on *Ocimum sanctum* @ 4gm/ of feed in the diet. All the haematological indices were within safety limits in this experiment. The normal PCV, Hb, WBC and with differential count of the white blood cells in the normal range is similar to the findings of Thange (2009) who observed non-significant effect of *Ocimum sanctum* and other herbal preparation on monocytes, lymphocytes, esinophils and heterophils. The normal values of the haematological indices indicate the nutritional status of the grower chicken and thus the adequate nourishment of the birds as reported by Church et al. (1984) in his study. This implies that the immune system of the birds was also functioning adequately. The numerical differences observed in haematological indices with the test ingredients supplemented diets suggest that

the diets were better utilized and assimilated into the blood stream for use by the birds. There were also no sign of anemia or ill-health as the degree of anemia is determined by Hb, PCV, and RBC count which were in the normal range in the entire course of the experiment. Thus our findings strongly agree with Nagalakshmi et al. (1996) and Gowda et al. (1998) who reported that bitter principles of medicinal plants possess a strong influence on haematological traits depending on their nutritional status.

The increased content of total protein, albumin, globulin, calcium, phosphorus and with decreased levels of enzymes AST, ALP with other paramaters within the normal range is in agreement with the findings of Zhang et al. (2009) who found higher total protein concentration at 21 and 42 days of age in broilers fed with ginger powder and also in accordance with Goerge et al. (2015) and Tietz (1976). According to Awosanya et al. (1999) blood protein depends on the quality and quantity of dietary protein available in the feed. Thus the quality and quantity of the dietary proteins were nutritionally adequate on adding these herbal test ingredients and there was no alteration of normal systemic protein utilization and there is no sign of anaemia in the groups as the total protein was in the normal range (Siegmund, 1973). The present results of increased total protein and significant increase in globulin in the test groups as compared to control, match with those obtained in broiler chicks (Abdo and Zeinb, 2004) to citric acid and acetic acid inclusion, respectively. These results indicate that supplemented herbal ingredients may improve the immune response as globulin level has been used as an indicator of immune responses and source of antibody production. El-Kerdawy (1996) stated that high globulin level signify better disease resistance and immune

response. This result is in harmony with those of (Rahmani and Speer, 2005) who found higher percentage of gamma globulin in broilers given organic acids than the control ones. Thus the enhancement of immune response associated with dietary acidification could be due to the inhibitory effects of the herbal ingredients against the pathogenic microorganisms throughout the GI-tract.

The increase of Ca and P levels in blood serum produced by addition of herbs may be attributed to the lowering of GI-tract pH by using these herbal ingredients, which increases the absorption of such minerals from the gut into the blood stream. Improving the utilization of calcium and phosphorus is in accordance with the findings of Boling et al. (2001) who noticed similar results on supplementing organic acids. Also, Abdo and Zeinb (2004) observed an increase in blood calcium of broiler chicks fed on dietary acidifier.

The reduction in the enzyme levels AST and ALP indicate the hepatoprotective nature of the herbal preparation as the plant extract could repair the hepatic injury and/or restore the cellular permeability thus reducing the liver toxicity and preventing enzyme leakage into the blood circulation (Mathivanan and Edwin, 2012) and proves that the herbal test ingredients are not toxic (Toppo et al. 2009; Ali and Ismail, 2012; Qureshi et al., 2015) to the birds on oral feeding and could tolerate the addition of the herbal ingredients at the dose of 0.5% without any deleterious effects on liver functions. These results are in full agreement with Abdel-Azeem et al. (2000) who observed reduced AST although ALT was not significantly affected.

Many researchers found that garlic exhibits hypoglycemic effect and proposed that garlic can act as anti-diabetic agent by either increasing the pancreatic secretion of insulin from beta cells or its release from bound insulin (Jain and Vyas, 1975). The principal active ingredients in garlic are believed to be allyl propyl disulphide and diallyl disulphide oxide (allicin). Abdul-Rahman (2012), Safaei et al. (2013) and Mamoun et al. (2014) reported that incorporation of dietary fenugreek seeds in broilers at 1% level significantly decreased the blood glucose levels. The reduction in the serum glucose levels may be related to direct  $\beta$ -cell stimulation by amino acid (4-hydroxy isoleucine) which increases insulin secretion thus improves glucose tolerance when fenugreek seeds are fed (Sauvaire et al., 1998; Schryver, 2002). Grodsky et al. (1965) found that there may be substances that cause antagonistic effect between garlic and natural insulin and such antagonistic action might have occurred in this experiment as there was significant increase of glucose in the higher dose group.

Thus the increased glucose in the higher dose group with increased ALT, both the values within the normal range with some discrepancies in the results may be due to the differences in the dose levels as well as the experimental period and the experimental conditions.

## CONCLUSION

It can be concluded that supplementation of the herbs *Ocimum sanctum* (Tulsi), *Allium sativum* (Garlic), *Curcuma longa* (curcumin), *Trigonella foenum graecum* (Fenugreek) and *Zingiber officinale* (ginger) could be important to improve layer performance as it is able to improve the immunity in the birds and increase the utilization of minerals required for an ideal production. The birds were found in a good health with no signs of anaemia which revealed the valuable and significant effects of these herbs.

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## Competing interests

The authors have no competing interests to declare.

## Consent to publish

The authors consent to publish the work.

## Author's contribution

All authors contributed significantly and equally in present article.

## REFERENCES

- Abdel-Rahman HA, Fathallah SI and Helal AA (2014). Effect of Turmeric (*Curcuma longa*), Fenugreek (*Trigonella foenum-graecum* L.) and/or bioflavonoid supplementation to the broiler chicks diet and drinking water on the growth performance and intestinal morphometric parameters. Global Vet, 12 (5): 627-635. DOI: 10.5829/idosi.gv.2014.12.05.83148
- Abdo M and Zeinb A (2004). Efficacy of acetic acid in improving the utilization of low protein-low energy broiler diets. Egyptian Poultry Science, 24: 123-141.
- Ajit S, Doley P, Neeraj and Prasad J (2014). Effect of dietary supplementation of tulsi (*Ocimum sanctum*) leaf powder on haematology and serum biochemistry

- of broiler chicks. *International Journal of Biological and Pharmaceutical Research*, 5 (1): 89 - 92.
- Ali AHH and Ismail ZSH (2012). Some physiological changes related to *Fenugreek* seeds supplementation to broiler chick's diet. *Egyptian Journal of Nutrition and feeds*, 15: 95-104. DOI: 10.3923/ijps.2015.664.669
- Ashish RS, Bajaj VK, Sekawat PS and Singh K (2013). Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Ocimum Sanctum* L. *Journal of Natural product and Plant Resources*, 3 (1): 51-58.
- Awosanya B, Joseph JR, Apata DF and Agbola MA (1999). Performance, blood chemistry and carcass quality attributes of rabbits fed raw and processed pueraria seed meal. *Tropical Journal of Animal Science*, 2(2): 89-96.
- Ayasan (2013). Effects of dietary inclusion of protexin (probiotic) on hatchability of Japanese Quails. *Indian Journal of Animal Science*, 83 (1): 78-81. <https://www.researchgate.net/publication/287696519>
- Basu SK, Acharya SN, Bandara MS, Friebe D and Thomas JE (2009). Effects of genotype and environment on seed and forage yield in fenugreek (*Trigonella foenum-graecum* L.) grown in western Canada. *Australian Journal of Crop Science, Southern Cross Journals*, 3 (6): 305-14. <https://pdfs.semanticscholar.org>
- Bhargava S, Dhabhai K, Batra A, Sharma A and Malhotra B (2012). *Zingiber officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. *Journal of Chemical and Pharmaceutical Research*, 4 (1): 360-364. <http://www.jocpr.com>
- Boling SD, Frankenbach JL, Snow CM, Parsons and Baker DH (2001). The effect of citric acid on the calcium and phosphorus requirements of chicks fed Corn - soybean meal diets. *Poultry science*, 80: 783-788. <https://oup.silverchair-cdn.com>
- Cardelle CA, Soria AC, Martinez MC and Villamiel M (2010). A comprehensive survey of garlic functionality. In: *Garlic consumption and Health*. Chapter 1, pp 1-60. <http://digital.csic.es/bitstream>
- Church JP, Judd JT, Yomg CW, Kebay TL and Kim WW (1984). Relationship among dietary constituents and specific serum clinical components of subjects eating self selected diets. *America Journal Clinical Nutrition*, 40: 1338- 1344.
- Deshmukh M V (2014). Investigation of antibacterial potential of turmeric (*Curcuma longa*) on enteric pathogens. *International Journal of Applied Research*, 4 (12): 556-558. <https://www.worldwidejournals.com>
- El-Kerdawy DMA (1996). Acidified feed for growing rabbits. *Egyptian Journal of Rabbit Science*, 6 (2): 143-156. <http://easterplanet.ca/>
- Gabriel I, Lessire M, Mallet S and Guillot JF (2006). Microflora of the digestive tract : Critical factors and consequences for poultry. *World's Poultry Science Journal*, 62:499–511.
- Gatne MM, Patil R, Ravikanth K, Shivi M and Rekle DS (2010). Evaluation of immunomodulatory effect of Stresroak premix in broiler chick. *Veterinary world*, 3 (3): 122-125. <http://www.veterinaryworld.org>
- George OS, Kaegon SG and Igbokwe AA (2015). Feed additive effects of graded levels of ginger (*zingiber officinale*) on serum metabolites of broilers. *Journal of Agriculture and veterinary Sciences*, 8 (3): 59 – 62. <http://www.iosrjournals.org>
- Gole MA (2001). Effect of liquid preparation of vitamin E and Selenium on growth and immune response of broilers. M.V.Sc. thesis submitted to Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.
- Gowda SK, Verma SVS, Elangovan AV and Singh AD (1998). Neem (*Azadirachta indica*) kernel meal in the diet of white leghorn layer. *British Poultry Science*, 39(5): 648-652. <https://www.ncbi.nlm.nih.gov>
- Grodsky GM, Karam JH and Forsham PH (1965). Production of autoantibodies to insulin in man and rabbits. *Diabetes*, 14: 396-403. <https://www.ncbi.nlm.nih.gov>
- Jain RC and Vyas CR (1975). Garlic in alloxan induced diabetic rabbits. *American Journal of Clinical Nutrition*, 28 (7):684-685. <https://www.ncbi.nlm.nih.gov>
- Janardhana V, Broadway MM, Bruce MP, Lowenthal JW, Geier MS, Hughes RJ and Bean AGD (2009). Prebiotics modulate immune responses in the gut-associated lymphoid tissues of chickens. *Journal of Nutrition*, 139 (7): 1404-1409. DOI: 10.3945/jn.109.105007
- Jeurissen SH, Lewis F, Van der Klis JD, Mroz Z , Rebel JM and Ter Huurne AA (2002). Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. *Current Issues in Intestinal Microbiology*, 3: 1–14. <http://www.scirp.org> ; ReferenceID=1733591
- Jinsuklee (2004). Medicinal Plants. A powerful health aid, Science creative quarterly. <http://www.seq.ubc.ca/medicinal-plants-a-powerful-health-aid/>, retrived on 15.04.13.

<http://www.scq.ubc.ca/medicinal-plants-a-powerful-health-aid/>

- Kabir SML (2009). The role of probiotics in the poultry industry. *International Journal of Molecular sciences*, 10(8): 3531- 3546. DOI: 10.3390/ijms10083531
- Kolte MV (1993). Effect of *Ocimum sanctum* linn., *Pyllanthus niruri* linn and active haematology, metabolic profile and immune response in goats. M. V. Sc., Thesis. (Unpub) Submitted to Dr. PDKV, Akola.
- Mamoun T, Mukhtar A and Mohamed HT (2014). Effect of Fenugreek seed powder on the Performance, Carcass Characteristics and Some Blood Serum Attributes. *Advance Research in Agriculture and Veterinary Science*, 1(1) : 6-11. <http://www.aravsjournal.com>
- Mathivanan R and Edwin SC (2012). Haematological and serum biochemical parameters of broilers fed with *Andrographis paniculata* as an alternative to antibiotic growth promoter. *Journal of Medicinal Plants Research*, 64 (44): 5647-5650
- Mitruka BM, Rawnsley HM, Vadehra BV (1977). Clinical biochemical and haematological reference values in normal experimental animals. Masson Publishing USA Inc. 272.
- Nagalakshmi D, Sastry VRB, Agrawal RC, Ketiyar DK and Verma SVS (1996). Performance of broiler chicks fed on alkali treated (*Azadirachta indica*) kernel cake as a protein supplement. *British Poultry Science*, 37 (4):809-818. DOI: 10.1080/00071669608417910
- Onu PN (2010). Evaluation of two herbal spices as feed additives for finisher broilers. *Biotechnology in Animal Husbandry*, 26 (5-6): 383-392. DOI: <http://dx.doi.org/10.2298/BAH1006383O>
- Pirgozliev V, Murphy TC, Owens B, George J and Mccann MEE (2008). Fumaric and sorbic acid as additives in broiler feed. *Research in Veterinary Science*, 84 (3):387-394. <https://www.ncbi.nlm.nih.gov>
- Qureshi S, Banday MT, Adil S, Shakeel I and Munshi ZH (2015). Effect of dandelion leaves and fenugreek seeds with or without enzyme addition on performance and blood biochemistry of broiler chicken and evaluation of their *in vitro* antibacterial activity. *Indian Journal of Animal Science*, 85 (11): 1248-1254. <https://www.researchgate.net/publication/287329961>
- Rahmani HR and Speer W (2005). Natural additives influence the performance and humoral immunity of broilers. *International Journal of Poultry Science*, 4 (9): 713-717. <http://agris.fao.org/ID=DJ2012051528>
- Reuter HD, Koch HP and Lawson LD (1996). Therapeutic effects and applications of garlic and its preparations. In: *Garlic: The science and therapeutic application of Allium sativum L. and Related Species*, Koch, H.P. and L.D. Lawson (Eds.). Williams and Wilkins, Baltimore, MD, 135 - 213.
- Rivlin RS (2001). Historical perspective on the use of garlic. *Journal of Nutrition*, 131 (35): 957 -954.
- Safaei A, Rahanjam SM and Gharajanlu M (2013). Effect of *Trigonella foenum-graecum* on immune response and some blood parameters of broilers. *Scholarly Journal of Agricultural Science* Vol. 3(4): 117-120. <http://www.scholarly-journals.com/SJAS>
- Sauvaire Y, Petit P and Broca C (1998). 4-Hydroxyisoleucine, A novel amino acid potentiator of Insulin secretion. *S Diabetes*, 47 (2): 206-210.
- Schryver T (2002). *Fenugreek*. *Total Health*, 24 (4): 42-44.
- Shukla ST, Kulkarni VH, Habbu PV, Jagadeesh KS, Patil BS and Smita DM (2012). Hepatoprotective and antioxidant activities of crude fractions of endophytic fungi of *Ocimum sanctum* Linn. in rats. *Oriental Pharmacy and Experimental Medicine*, 12 (2): 81-91. DOI: 10.1007/s13596-012-0061-7
- Siegmund OH (1973). The Merck veterinary manual, 4th Ed., Merck & Co. INC., Rahway, N.J., USA.
- Sojoudi MR, Dadashbeiki M and Bouyeh M (2012). Effects of different levels of symbiotic TechnoMos on broiler performance. *Research Opinions in Animal and Veterinary Sciences*, 2(4), 243-248. <https://www.researchgate.net>
- SPSS (2009). Computer software SPSS Inc. Headquarters, Wacker Drive, Chicago, Illinois-U.S.A.
- Thange HP(2009). Efficacy of different herbal preparation in broiler diet for immunomodulation. Thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur.
- Thatte UM and Dhakanukar SA (1989). Immunotherapeutic modification of experimental infection by Indian medicinal Plants. *Phytotherapy Research*, 3: 43 - 49.
- Tietz NW (1976). *Fundamentals of clinical chemistry*, 2<sup>nd</sup> edition.W.B. Saunders Company Ltd., Philadelphia.pp.246.
- Tipu MA, Akhtar MS, Anjum MI and Raja ML (2006). New dimensions of medicinal plants as animal feed. *Pakistan Veterinary Journal*, 26(3): 144-148. <http://pvj.com.pk>
- Toppo FA, Rachna A and Pathak AK (2009). Pharmacological actions and potential uses of *Trigonella foenum graecum*: A Review. *Asian Journal of Pharmaceutical and Clinical Research*, 2 (4): 69-74. <http://ajpcr.com>
- Vijayalakshmi K, Priya V and Jananic RK (2011). GC/MS determination of bioactive components of *Trigonella*

- foenum grecum*. Journal of chemical and pharmaceutical Research, 3(5): 35 -40.
- Virupaksha JH, Shivakumar H and Jayakumar BHMS (2007). Evaluation of anti-diarrhoeal activity of fenugreek seed extracts in rats. Pharmacologyonline., 2; 255- 266. <http://pharmacologyonline.silae.it/>
- Vivian UOO, Ndofo-Foleng HM, Olorunleke SO and Uguru JO (2015). Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. Journal of Agricultural Sciences, 7 (4): 167 - 173. <http://citeseerx.ist.psu.edu>
- Yang Y, Iji PA, Kocher A, Thomson E, Mikkelsen LL and Chot M (2008). Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilization, nutrient digestibility and intestinal microflora. British Poultry Science, 49: 186-194.
- YangY, Iji PA and Choct M (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World Poultry Science Journal, 65 pp. 97 - 114. DOI:10.1017/S0043933909000008
- Zhang GF, Yang ZB, Wang Y, Yang WR, Ziang SZ and Gai GS (2009). Effects of Ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status and serum metabolites of broiler chickens. Poultry science, 88: 2159 - 2166. DOI: 10.3382/ps.2009-00165.





# Effects of Dietary Inclusion of Probiotics and Organic Acids on Performance, Intestinal Microbiology, Serum Biochemistry and Carcass Traits of Broiler Chickens

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## ABSTRACT

This study was conducted to evaluate the effects of probiotics and organic acids, as alternative feed additives to antibiotics, on productive performance of broilers. Two different types of probiotics varying in the microbial content were tested and organic acids blend was compared against a single organic acid (lactic acid). One hundred and ninety eight broiler chicks were randomly allocated into six treatments, each with 33 chicks. Every treatment consisted of 3 replicates with 11 birds per replicate. The dietary treatments were a control diet without any feed additives or the same control diet supplemented either with a commercial antibiotic (Maxus<sup>®</sup>G200), probiotics (Bactocell<sup>®</sup> or Biopellet-S<sup>®</sup>) or acidifiers (Salmo-Nil Dry<sup>®</sup> or lactic acid). The antibiotic was added to the diet at the rate of 0.005%, whereas the probiotics were used at 0.01%. The product Salmo-Nil Dry<sup>®</sup> was provided to the diet at a level of 0.4%, whereas the lactic acid was used at 0.20 %. It was found that the antibiotic, probiotics and lactic acid increased the body weight. All dietary supplements improved the FCR compared to the control. The additives reduced the serum cholesterol level and the pH of small intestine but did not affect the carcass yield, breast or organ weights. The feed supplements showed a numerical decrease in intestinal aerobes, fecal coliforms and *E. coli* counts. In addition, all additives significantly reduced total aerobic and staphylococcus counts in the carcass meat, with a numerical decrease in *E. coli* count. In conclusions, probiotics and acidifiers can be used as potential alternatives to antibiotics in broiler diets. No difference between the used types of probiotics was detected. Lactic acid alone seems to produce better performance results than the organic acid mixture. The effect of lactic acid produced by bacteria might be similar to that of the chemical one.

**Key words:** Broilers, Probiotic, Organic acids, Performance, Lactic acid, Carcass

## INTRODUCTION

The efficiency of poultry digestion depends on the microorganisms which live naturally in the digestive tract. Certain feed additives can be added to the diet to create favourable conditions in the intestinal tract for the digestion of feed. Antibiotics have been extensively used in poultry diets to control diseases and improve the productive performance. However, the use of antibiotics resulted in several complications such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the birds' body (Burgat, 1999), and imbalance

of normal intestinal microflora (Andremont, 2000). Therefore, many countries in Europe have been banned the antibiotics usage as feed additives. As a result, there is an increasing interest in finding alternatives to antibiotics in poultry industry. Among these alternatives are the use of probiotics and organic acids in the animal nutrition. These additives are generally recognized as safe and are commonly used in recent years.

Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). These additives

are acting through maintaining a beneficial microbial population by competitive exclusion and antagonism (Fuller, 1989; Katoch et al., 2017), enhancing feed intake and digestion (Nahanshon et al., 1993; Hossein et al., 2017), and modifying bacterial metabolism (Jin et al., 1997; Pourakbari et al., 2016). Lactobacilli and enterococci are among the wide variety of microbial species that have been used extensively in poultry diets as probiotics (Patterson and Burkholder, 2003). The feeding of probiotics has been reported to improve growth performance and feed efficiency in broiler chickens (Kabir et al., 2004; Mountzouris et al., 2007; Samli et al., 2007, Awad et al., 2009; Tabidi et al., 2013; Nawaz et al., 2016).

The organic acids have antimicrobial activity as they are undissociated and can penetrate the bacterial cell wall and upset the growth of certain types of bacteria (Dhawale, 2005). Additionally, these acids can diminish the pH values of digesta and have trophic impacts on the mucosa of digestive tract (Dibner and Buttin, 2002). Furthermore, organic acids supplementations have been found to reduce colonization of pathogens on the intestinal wall and production of bacterial toxins, thus preventing the damage to the intestinal epithelial cells (Langhout, 2000). These acids can also improve the digestibility of protein and minerals such as Ca, P, Mg and Zn (Kirchgessner and Roth, 1988; Waseem Mirza et al., 2016). The use of organic acids and probiotics has been reported to protect the chicks by competitive exclusion (La Ragione and Woodward, 2003; Hassan et al., 2010), increase the nutrient utilization and performance (Denli et al., 2003; Adil et al., 2010; Khan and Iqbal, 2016).

Therefore, the present study was conducted to evaluate the effects of probiotics and organic acids, as alternative feed additives to antibiotics, on productive performance of broilers. Furthermore, two different types of probiotics varying in the microbial content were tested. The first probiotic type consisted of *Pediococcus acidilactici* bacteria, whereas the second one composed of *Bacillus subtilis* and *Enterococcus faecium*. In addition, a commercial acidifier product, which consists of organic acids blend, was investigated against a single organic acid (lactic acid). Moreover, the impacts of natural lactic acid produced by “probiotic” bacteria (*Pediococcus acidilactici*) were compared with the “chemical” lactic acid per se.

## MATERIALS AND METHODS

### Birds and diets

One hundred and ninety eight, one-day-old, broiler chicks (Cobb 500) were obtained from a local commercial

hatchery. The birds were randomly allocated into six treatments, each with 33 chicks. Every treatment consisted of 3 replicates with 11 birds per replicate. The chicks were housed in pens (1.10 x 1.0 m<sup>2</sup> per replicate pen) with a bedding of wood shavings. The experiment lasted for 42 days. The initial brooding temperature was 33°C in the first week of age and reduced gradually 2°C per week until reaching about 20 °C at the end of experiment. A lightening period of 23 h per day was provided throughout the experimental period. The dietary treatments were a control diet without any feed additives or the same control diet supplemented either with a commercial antibiotic (Maxus<sup>®</sup>G200), probiotics (Bactocell<sup>®</sup> or Biopellet-S<sup>®</sup>) or acidifiers (Salmo-Nil Dry<sup>®</sup> or lactic acid). The antibiotic (Elanco Animal Health, USA) contained 200g of avilamycin activity per kg. Both types of probiotics were different and varied in the microbial composition. Bactocell<sup>®</sup> (Lallemand S.A.S, France) consisted of lactic acid producing bacteria, *Pediococcus acidilactici* 1.0 x 10<sup>10</sup> CFU /g, and dextrose as a carrier up to 1g, while Biopellet-S<sup>®</sup> (Samu Median Co., LTD, Korea) comprised of *Bacillus subtilis* 3.0 x 10<sup>10</sup> CFU and *Enterococcus faecium* 3.0 x 10<sup>10</sup> CFU per kg, and dextrose up to 1 kg. Also, two different kinds of acidifiers were used; Salmo-Nil Dry<sup>®</sup> (Nutri- AD International, Belgium) which is a commercial by-product containing a group of acids ( Ca – formate 60%, Ca – propionate 10%, Ca - lactate 10%, Ca - citrate 20%), whereas the second one consisted of one type of acid (lactic acid powder-food grade 88%, ICIS, UK).The antibiotic was added to the diet at the rate of 0.005%, whereas the probiotics were used at 0.01%. The product Salmo-Nil Dry<sup>®</sup> was provided to the diet at a level of 0.4%, whereas the lactic acid was used at 0.20 %.The dietary doses of the tested feed additives, except lactic acid, were used according to the recommended levels of the produced companies.

During the experiment, the birds were fed on a starter diet for 21 days, and then switched to a grower diet from day 22 up to day 42. The diets were calculated to meet or exceed the nutrient requirements for broiler chickens recommended by NRC for poultry (1994). The control and experimental diets were formulated to have the same nutrient contents. The experimental diets were supplemented without (control) or with the tested feed additives. The antibiotic and probiotics were added to the diets in very small proportions by substituting equal amounts of corn, while the acidifier diets were formulated by adjusting the amounts of corn, vegetable oil and soybean meal (SBM) to maintain the energy density and protein level. Ingredients and chemical composition of the diets are shown in Table 1 and 2. The used ingredients

were analyzed for their proximate composition using the standard laboratory methods according to AOAC (2005). The diets were formulated based on the nutrient contents of the ingredients. Feed and water were offered to the birds *ad libitum* during the experiment.

### Ethical approval

All animal procedures were approved by the Animal Ethics Committee at Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

**Table 1.** Physical and chemical composition (%) of the starter diets (as fed)

Composition	Group					
	Control	Antibiotic	Probiotics		Acidifiers	
	( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid
<b>Dietary ingredients</b>						
Yellow corn	46.93	46.925	46.92	46.92	46.06	46.49
Soybean meal	36.00	36.00	36.00	36.00	36.17	36.09
Sunflower oil	7.08	7.08	7.08	7.08	7.38	7.23
Corn gluten	6.00	6.00	6.00	6.00	6.00	6.00
Dicalcium phosphate	1.70	1.70	1.70	1.70	1.70	1.70
Limestone	1.41	1.41	1.41	1.41	1.41	1.41
Common salt	0.47	0.47	0.47	0.47	0.47	0.47
DL- methionine	0.11	0.11	0.11	0.11	0.11	0.11
Vit. and min. premix <sup>1)</sup>	0.30	0.30	0.30	0.30	0.30	0.30
Feed additives	-	0.005	0.01	0.01	0.40	0.20
<b>Chemical composition</b>						
Metabolizable energy, kcal/kg	3200.0	3199.8	3199.6	3199.6	3200.1	3200.0
Dry matter	91.39	91.39	91.38	91.38	91.07	91.23
Crude protein	23.0	23.0	23.0	23.0	23.0	23.0
Methionine	0.51	0.51	0.51	0.51	0.51	0.51
Methionine + Cystine	0.90	0.90	0.90	0.90	0.90	0.90
Lysine	1.15	1.15	1.15	1.15	1.15	1.15
Ether extract	9.43	9.43	9.43	9.43	9.71	9.57
Crude fiber	3.13	3.13	3.13	3.13	3.13	3.13
Ash	6.39	6.39	6.39	6.39	6.40	6.40
Calcium	1.00	1.00	1.00	1.00	1.00	1.00
Phosphorus, available	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20	0.20	0.20

<sup>1)</sup> Vitamins and minerals premix (Agri-Vet Company, Egypt): each 3.0 kg contain Vit. A, 12000000 IU; Vit.D<sub>3</sub> 2000000 IU; Vit.E, 10000 mg; Vit.K<sub>3</sub>, 2000 mg; Vit.B<sub>1</sub>, 1000 mg; Vit.B<sub>2</sub>, 5000 mg; Vit. B<sub>6</sub>, 1500 mg; Vit.B<sub>12</sub>, 10mg; biotin, 50mg; pantothenic acid, 10000 mg; nicotinic acid, 30000 mg; folic acid, 1000 mg; choline chloride, 250000 mg; Mn, 60000 mg; Zn, 50000 mg; Fe, 30000 mg; Cu, 10000 mg; I, 1000 mg; Se, 100mg; Co, 100mg and complete to 3.0 kg by calcium carbonate.

**Table 2.** Physical and chemical composition (%) of the grower diets (as fed)

Composition	Group					
	Control	Antibiotic	Probiotics		Acidifiers	
	( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid
<b>Dietary ingredients</b>						
Yellow corn	53.35	53.345	53.34	53.34	52.52	52.96
Soybean meal	36.15	36.15	36.15	36.15	36.29	36.20
Sunflower oil	7.14	7.14	7.14	7.14	7.43	7.28
Limestone	1.46	1.46	1.46	1.46	1.46	1.46
Dicalcium phosphate	1.18	1.18	1.18	1.18	1.18	1.18
Common salt	0.35	0.35	0.35	0.35	0.35	0.35
DL- methionine	0.07	0.07	0.07	0.07	0.07	0.07
Vit. and min. premix <sup>1)</sup>	0.30	0.30	0.30	0.30	0.30	0.30
Feed additives	-	0.005	0.01	0.01	0.40	0.20
<b>Chemical composition</b>						
Metabolizable energy, kcal/kg	3200.3	3200.1	3199.9	3199.9	3200.2	3200.2
Dry matter	91.21	91.20	91.20	91.20	90.88	91.04
Crude protein	20.01	20.01	20.01	20.01	20.00	20.00
Methionine	0.39	0.39	0.39	0.39	0.39	0.39
Methionine + Cystine	0.73	0.73	0.73	0.73	0.73	0.73
Lysine	1.11	1.11	1.11	1.11	1.11	1.11
Ether extract	9.58	9.58	9.58	9.58	9.84	9.70
Crude fiber	3.20	3.20	3.20	3.20	3.19	3.20
Ash	5.80	5.80	5.80	5.80	5.80	5.80
Calcium	0.90	0.90	0.90	0.90	0.90	0.90
Phosphorus, available	0.35	0.35	0.35	0.35	0.35	0.35
Sodium	0.15	0.15	0.15	0.15	0.15	0.15

<sup>1)</sup> Vitamins and minerals premix (Agri-Vet Company, Egypt): each 3.0 kg contain Vit. A, 12000000 IU; Vit.D<sub>3</sub> 2000000 IU; Vit.E, 10000 mg; Vit.K<sub>3</sub>, 2000 mg; Vit.B<sub>1</sub>, 1000 mg; Vit.B<sub>2</sub>, 5000 mg; Vit. B<sub>6</sub>, 1500 mg; Vit.B<sub>12</sub>, 10mg; biotin, 50mg; pantothenic acid, 10000 mg; nicotinic acid, 30000 mg; folic acid, 1000 mg; choline chloride, 250000 mg; Mn, 60000 mg; Zn, 50000 mg; Fe, 30000 mg; Cu, 10000 mg; I, 1000 mg; Se, 100mg; Co, 100 mg and complete to 3.0 kg by calcium carbonate.

### Growth performance

The diets were offered to the chicks daily and feed intake/day was calculated after removal of the refused feed. The total feed consumption per day was divided by the number of birds in each pen to obtain the average daily feed intake / bird. All the birds were individually weighed at the start and end of the experiment as well as at weekly intervals throughout the experiment. Accordingly, the weekly weight gain of the birds was measured. Based on the feed intake and weight gain, the feed conversion ratio was estimated and corrected for mortality on a bird day basis. The mortality rate was recorded daily throughout the experiment.

### Excreta quality

The quality of excreta from each dietary treatment was evaluated by measuring its dry matter (DM) content. The excreta of six birds per treatment (two birds / replicate) were collected at the end of starter (d 21) and grower (d 42) periods. The collected fresh excreta from each bird were taken, thoroughly mixed and then dried at 105 °C in hot air oven for 24h to determine the DM content.

### Blood parameters

Blood samples of six birds per treatment (two birds /replicate) were collected at the end of starter (d 21) and grower (d 42) periods. The birds were sacrificed and the

samples of blood were taken from the neck of birds and then collected in blood tubes. The samples were centrifuged at 3.000 rpm for 15 minutes for separating the serum. Afterwards, the obtained serum was stored at – 20°C until analysis. The serum samples were analyzed for some biochemical parameters, including glutamic pyruvic transaminase (GPT), glutamic oxaloacetate transaminase (GOT), creatinine, glucose and cholesterol by using chemical kits.

#### **pH values of intestinal contents**

Immediately after sacrificing the selected birds (at day 21 and 42) for obtaining the blood samples, the digesta of small intestine and caecum of only three birds / treatment (one bird per replicate) were individually isolated in tubes. These samples were diluted with water at the rate of 1:5, and then thoroughly mixed. Thereafter, the samples were measured for pH values using pH meter (Youssef et al., 2012).

#### **Carcass characteristics:**

Six birds from each treatment (two birds / replicate), close to the average live body weight, were selected at the end of experiment. Birds were weighed, subjected to 24h-feed withdrawal with free access to water, reweighed and slaughtered by neck cutting. The birds were scalded, defeathered, and eviscerated after removal of head, neck and legs. The carcass without giblets was weighed, expressed as a percentage of its live weight and considered as the carcass yield. In addition, the weight of the breast, proventriculus, gizzard, liver and heart was recorded and its relation to the live body weight of the birds, in percentages, was calculated.

#### **Microbiological examination**

##### ***Intestinal digesta***

At the end of the experiment, the contents of small intestine (mixed contents of duodenum, jejunum and ileum) and caecum of 3 birds / treatment were individually collected directly after slaughter in separate sterile Petri dishes. Afterwards, one gram from each sample was mixed with 9 ml of 0.1% sterile peptone water and then ten- fold serial dilution up to  $10^{-6}$  was prepared. One ml from each serial dilution of intestinal contents was separately pipetted into double set of Petri dishes and mixed with 15 ml of melted deMan, Rogosa and Sharpe (MRS) agar (Biolife) then incubated at 42°C under microaerophilic conditions (5% CO<sub>2</sub>) for 48h for lactobacilli count; another 1 ml was mixed with 15 ml of melted standard plate count agar (SPCA, Oxoid, CM325) and incubated at 35°C for 48h for total aerobic bacterial

count; another 1 ml was inoculated into three replicate tubes of Lauryl Sulphate Tryptose Broth (LST, Oxoid, CM451) with inverted Durham's tubes and incubated at 35°C for 48 hours for determination of the most probable number (MPN) of coliforms. A loopful from each positive LST tubes showing gas was transferred into tubes containing Brilliant Green Bile Lactose broth (Oxoid, CM31) with inverted Durham's tubes and incubated at 35°C for 48 hours. Positive tubes showing gas production were recorded and MPN of coliforms was estimated. A loopful from each positive brilliant green bile lactose broth was inoculated into tubes of *E. coli* broth (Biolife, 401425), and then incubated at 44°C for 48 hours for determination of MPN of faecal coliforms. Positive tubes showing gas production were calculated as MPN of faecal coliforms. A loopful from each positive *E. coli* broth tubes was streaked onto plates of eosin methylene blue agar (Oxoid, CM69) and incubated at 35°C for 24 hours for determination of MPN of *E. coli*. Typical colonies appear as greenish metallic nucleated with dark purple center with or without sheen.

##### ***Carcass meat***

After slaughtering and dressing of broiler chickens at the end of experiment, 3 birds per treatment (one bird / replicate) were used for microbiological examination of the muscles. The muscle samples were prepared according to the muscle maceration technique recommended by ICMSF (1978). The muscle surface was sterilized by hot spatula, and then ten grams of breast and thigh muscles (5 g from each) were taken from deep muscle under aseptic conditions. Then, the samples were transferred to a sterile homogenizing jar to which 90 ml of 0.1 % sterile peptone water were added. The contents were thoroughly homogenized for 2 minutes at 2000 r.p.m. using a sterile homogenizer. Such homogenate was serially diluted as in the intestinal contents. Total aerobic, coliform, faecal coliform and *E. coli* counts were estimated as previously mentioned for intestinal samples. *Staphylococcus aureus* count was done according to APHA (1992) by spreading 100 µl from each dilution over a dry surface of Baird-Parker medium (BP, Oxoid, CM275) plates. Inoculated plates were incubated at 35°C for 24 hours. Suspected colonies were recorded and *Staphylococcus aureus* count was calculated.

##### **Statistical analyses**

The results were analysed statistically using Statistical Package for Social Science (SPSS for Windows (IBM), version 20, Chicago, USA, 2011). The data were analysed by using one-way ANOVA and subsequent



Duncan's multiple range test to determine the differences between the treatments. Results are expressed as means  $\pm$  SEM. Probability values of less than 0.05 ( $P < 0.05$ ) were considered significant.

## RESULTS

The antibiotic, probiotics and lactic acid increased significantly ( $P < 0.05$ ) the body weight during the grower period (3-6 weeks) compared to the control, but had no significant effect ( $P > 0.05$ ) on the birds' weight during the starter period (0-3 week). However, salmo-nil treatment did not influence the body weight comparing to the control group throughout the experiment.

During the starter period, the feed intake and weight gain were not affected by the dietary treatments (Table 3). Nevertheless, the feed conversion ratio (FCR) for birds fed diets containing the tested feed additives (1.44) was lower ( $P < 0.001$ ) than the control (1.56). Among the tested additives, the biopellet-s group was found to have the lowest FCR (1.39), whereas the highest one (1.49) was in the salmo-nil treatment. No difference ( $P > 0.05$ ) in FCR was detected between the birds fed diets supplemented with bactocell and acidifiers (salmo-nil and lactic acid). The mortality rate was lower in biopellet-s and acidifiers groups (3.03%) than the other treatments (6.06%).

During the grower period, the feed intake was lower in biopellet-s and acidifiers treatments than the control while that of other groups was not affected. Compared to the control, the weight gain of birds fed diets supplemented with antibiotic and bactocell was higher ( $P < 0.05$ ), but did not differ in other treatments. However, the weight gain in biopellet-s and lactic acid groups was similar to that of antibiotic and bactocell treatments. The FCR in all feed additives supplemented groups (about 1.93) was lower ( $P < 0.05$ ) than the control group (2.16). Moreover, there was no difference ( $P > 0.05$ ) in FCR between these additives. The mortality rate was null in control and acidifiers groups, but was about 3.20% in other treatments.

All over the experimental period, it was found that the feed intake, as in the grower period, was lower ( $P < 0.05$ ) in biopellet-s and acidifiers groups compared to the control. The weight gain of birds fed diets supplemented with antibiotic, probiotics and lactic acid was similar ( $P > 0.05$ ) and higher ( $P < 0.05$ ) than the control birds. However, no difference in the weight gain between salmo-nil and control was found. Moreover, the feed additives improved ( $P < 0.05$ ) the feed conversion ratio compared to the control (1.78 vs. 1.97). However, there was no difference ( $P > 0.05$ ) in FCR between the feed

additive groups. The mortality rate was 3.03% in acidifiers, 6.06% in control and biopellet-s, and 9.09% in antibiotic and bactocell groups. The excreta of the birds were analysed for the DM content at the end of starter and grower periods. There were no differences ( $P > 0.05$ ) in DM values between the different dietary treatments, indicating that the dietary supplements did not affect the excreta quality.

Supplementation of the feed additives exhibited no significant ( $P > 0.05$ ) differences in the serum concentration of SGPT, SGOT, creatinine, glucose, and cholesterol at the end of starter period (Table 4). The same findings in the serum constituents were found at the end of grower phase, with exception of cholesterol level which was lower ( $P < 0.05$ ) in all birds fed the dietary supplements compared with those fed the control diet.

The effect of dietary treatments on the pH values of the intestinal contents is presented in table 5. It was found that supplementation of antibiotic and salmo-nil significantly ( $P < 0.05$ ) reduced the pH of small intestine contents (5.84) at the starter period, but with insignificant ( $P > 0.05$ ) decrease in other dietary supplements (6.07) when compared with the control (6.29). Moreover, the feed additives numerically decreased the pH values (6.06 vs. 6.59) of small intestine at the grower period. However, the dietary additives did not influence ( $P > 0.05$ ) the pH of the caecum digesta at the starter or grower period compared to the control.

The carcass characteristics of the birds fed different diets are demonstrated in table 6. The carcass yield percentage did not show any significant differences ( $P > 0.05$ ) among the dietary treatments, but exhibited a numerical increase in probiotics (72.84 %), followed by antibiotic and lactic acid groups (about 71.45%) compared to the control (70.35%). Moreover, the relative weights of breast, proventriculus, gizzard, liver and heart were not affected ( $P > 0.05$ ) by the dietary supplements when compared with the control.

No significant ( $P > 0.05$ ) effect of dietary treatments on the intestinal bacterial count was observed (Table 7). However, the feed additives numerically decreased the total aerobic, coliforms, faecal coliforms and *E. coli* counts in both small intestine and caecum. On the other hand, there was a numerical increase in *Lactobacilli* count in acidifiers and probiotics, but it decreased in the antibiotic group. Concerning the microbial examination of carcass meat, all additives significantly ( $P < 0.001$ ) reduced the total aerobic and *Staphylococcus* counts (Table 8). Moreover, a numerical decrease in *E. coli* count in all treatments was noticed.

**Table 3.** Growth performance and excreta DM content of broilers fed different dietary treatments throughout the experiment

Period	Group						P-value
	Control	Antibiotic	Probiotics		Acidifiers		
	( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Starter period (0-3 wk)							
Feed intake, g	832.68 <sup>a</sup> ±23.24	830.62 <sup>a</sup> ±11.44	821.38 <sup>a</sup> ±23.25	814.16 <sup>a</sup> ±19.70	803.26 <sup>a</sup> ±3.87	802.52 <sup>a</sup> ±7.82	0.693
Weight gain, g	535.42 <sup>a</sup> ±24.83	582.60 <sup>a</sup> ±11.00	559.04 <sup>a</sup> ±18.34	587.41 <sup>a</sup> ±21.33	540.00 <sup>a</sup> ±3.36	556.97 <sup>a</sup> ±8.92	0.212
FCR, g/g	1.56 <sup>a</sup> ±0.03	1.43 <sup>cd</sup> ±0.01	1.47 <sup>bc</sup> ±0.01	1.39 <sup>d</sup> ±0.02	1.49 <sup>b</sup> ±0.002	1.44 <sup>bc</sup> ±0.01	0.0001
Excreta DM content,%	14.69 <sup>a</sup> ±1.49	15.59 <sup>a</sup> ±1.32	15.52 <sup>a</sup> ±2.09	15.37 <sup>a</sup> ±1.67	16.29 <sup>a</sup> ±0.69	15.28 <sup>a</sup> ±1.72	0.981
Mortality, %	6.06	6.06	6.06	3.03	3.03	3.03	
Grower period (3-6 wk)							
Feed intake, g	2513.8 <sup>a</sup> ±69.89	2457.0 <sup>ab</sup> ±31.88	2464.7 <sup>ab</sup> ±12.85	2375.7 <sup>b</sup> ±25.22	2263.5 <sup>c</sup> ±11.15	2265.4 <sup>c</sup> ±7.93	0.001
Weight gain, g	1163.3 <sup>bc</sup> ±16.17	1265.0 <sup>a</sup> ±13.36	1278.3 <sup>a</sup> ±39.14	1231.1 <sup>ab</sup> ±19.50	1143.0 <sup>c</sup> ±16.24	1208.4 <sup>ab</sup> ±19.98	0.006
FCR, g/g	2.16 <sup>a</sup> ±0.12	1.94 <sup>b</sup> ±0.06	1.93 <sup>b</sup> ±0.07	1.93 <sup>b</sup> ±0.02	1.98 <sup>b</sup> ±0.03	1.87 <sup>b</sup> ±0.04	0.011
Excreta DM content,%	12.08 <sup>a</sup> ±2.07	12.52 <sup>a</sup> ±1.14	14.38 <sup>a</sup> ±1.13	13.63 <sup>a</sup> ±1.40	13.99 <sup>a</sup> ±0.63	13.68 <sup>a</sup> ±1.16	0.830
Mortality, %	0.0	3.23	3.23	3.13	0.0	0.0	
Total period (0-6 wk)							
Feed intake, g	3346.4 <sup>a</sup> ±93.13	3287.7 <sup>ab</sup> ±20.44	3286.1 <sup>ab</sup> ±10.40	3189.8 <sup>bc</sup> ±44.92	3066.8 <sup>c</sup> ±7.28	3067.9 <sup>c</sup> ±15.75	0.002
Weight gain, g	1698.7 <sup>b</sup> ±8.67	1847.6 <sup>a</sup> ±24.36	1837.3 <sup>a</sup> ±20.80	1818.5 <sup>a</sup> ±40.82	1683.0 <sup>b</sup> ±12.89	1765.4 <sup>a</sup> ±28.90	0.002
FCR, g/g	1.97 <sup>a</sup> ±0.05	1.78 <sup>b</sup> ±0.04	1.79 <sup>b</sup> ±0.03	1.75 <sup>b</sup> ±0.02	1.82 <sup>b</sup> ±0.01	1.74 <sup>b</sup> ±0.03	0.001
Mortality, %	6.06	9.09	9.09	6.06	3.03	3.03	

<sup>a, b, c</sup> Means within the same row with different superscripts are significantly different (P< 0.05).

**Table 4.** Serum constituents of broiler chickens fed different diets at the end of starter (d 21) and grower (d 42) periods

Period	Parameter	Group						P-value
		Control	Antibiotic	Probiotics		Acidifiers		
		( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Starter	SGPT (μ/L)	5.33 <sup>a</sup> ±0.33	6.00 <sup>a</sup> ±0.58	4.33 <sup>a</sup> ±0.33	4.00 <sup>a</sup> ±0.58	5.00 <sup>a</sup> ±1.15	5.67 <sup>a</sup> ±0.67	0.312
	SGOT (μ/L)	187.7 <sup>a</sup> ±15.81	174.0 <sup>a</sup> ±31.77	211.0 <sup>a</sup> ±39.80	187.7 <sup>a</sup> ±11.35	173.3 <sup>a</sup> ±13.93	167.7 <sup>a</sup> ±3.38	0.794
	Creatinine (mg/dL)	0.28 <sup>a</sup> ±0.02	0.34 <sup>a</sup> ±0.02	0.36 <sup>a</sup> ±0.02	0.28 <sup>a</sup> ±0.01	0.34 <sup>a</sup> ±0.08	0.37 <sup>a</sup> ±0.07	0.659
	Glucose (mg/dL)	238.7 <sup>a</sup> ±21.18	230.0 <sup>a</sup> ±12.58	239.7 <sup>a</sup> ±22.15	236.3 <sup>a</sup> ±21.84	232.3 <sup>a</sup> ±12.67	223.3 <sup>a</sup> ±21.31	0.989
	Cholesterol (mg/dL)	142.3 <sup>a</sup> ±12.17	121.0 <sup>a</sup> ±34.77	142.3 <sup>a</sup> ±12.17	123.7 <sup>a</sup> ±17.70	140.0 <sup>a</sup> ±11.24	140.3 <sup>a</sup> ±13.68	0.919
Grower	SGPT (μ/L)	4.60 <sup>a</sup> ±0.51	3.80 <sup>a</sup> ±0.37	5.00 <sup>a</sup> ±0.45	4.40 <sup>a</sup> ±0.51	3.80 <sup>a</sup> ±0.37	5.00 <sup>a</sup> ±0.55	0.274
	SGOT (μ/L)	291.3 <sup>a</sup> ±5.85	258.8 <sup>a</sup> ±15.73	268.5 <sup>a</sup> ±16.42	264.2 <sup>a</sup> ±18.52	257.5 <sup>a</sup> ±29.94	284.2 <sup>a</sup> ±22.97	0.793
	Creatinine (mg/dL)	0.42 <sup>a</sup> ±0.04	0.32 <sup>a</sup> ±0.01	0.32 <sup>a</sup> ±0.02	0.41 <sup>a</sup> ±0.04	0.37 <sup>a</sup> ±0.03	0.42 <sup>a</sup> ±0.05	0.143
	Glucose (mg/dL)	196.2 <sup>a</sup> ±16.55	216.0 <sup>a</sup> ±8.01	208.0 <sup>a</sup> ±8.76	170.5 <sup>a</sup> ±18.34	217.4 <sup>a</sup> ±18.05	167.5 <sup>a</sup> ±21.06	0.161
	Cholesterol (mg/dL)	175.8 <sup>a</sup> ±14.41	112.6 <sup>b</sup> ±11.33	123.0 <sup>b</sup> ±17.68	101.0 <sup>b</sup> ±16.94	111.0 <sup>b</sup> ±8.67	116.8 <sup>b</sup> ±9.31	0.010

<sup>a, b</sup> Means within the same row with different superscripts are significantly different (P< 0.05).

**Table 5.** pH values of intestinal contents of birds fed different diets at the end of starter (d 21) and grower (d 42) periods

Period	Intestinal Segment	Group						P-Value
		Control	Antibiotic	Probiotics		Acidifiers		
		( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Starter	Small intestine	6.29 <sup>a</sup> ± 0.06	5.80 <sup>c</sup> ± 0.04	6.04 <sup>abc</sup> ± 0.01	6.13 <sup>ab</sup> ± 0.05	5.88 <sup>bc</sup> ± 0.19	6.03 <sup>abc</sup> ± 0.01	0.021
	Caecum	7.02 <sup>a</sup> ± 0.08	6.56 <sup>a</sup> ± 0.07	7.44 <sup>a</sup> ± 0.39	6.99 <sup>a</sup> ± 0.08	7.25 <sup>a</sup> ± 0.43	6.45 <sup>a</sup> ± 0.33	0.164
Grower	Small intestine	6.59 <sup>a</sup> ± 0.19	6.12 <sup>a</sup> ± 0.04	6.00 <sup>a</sup> ± 0.11	5.96 <sup>a</sup> ± 0.25	6.05 <sup>a</sup> ± 0.05	6.16 <sup>a</sup> ± 0.06	0.073
	Caecum	6.60 <sup>a</sup> ± 0.20	7.33 <sup>a</sup> ± 0.17	7.20 <sup>a</sup> ± 0.35	6.89 <sup>a</sup> ± 0.49	7.27 <sup>a</sup> ± 0.12	6.76 <sup>a</sup> ± 0.33	0.329

<sup>a, b, c</sup> Means within the same row with different superscripts are significantly different (P< 0.05).

**Table 6.** Carcass and organ weights relative to BW (%) of broiler chickens fed different experimental diets

Parameter	Group						P-value
	Control	Antibiotic	Probiotics		Acidifiers		
	( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Carcass yield	70.35±0.89	71.24±0.81	73.03±0.96	72.65±0.29	70.81±0.80	71.66±0.94	0.186
Breast	22.03±0.79	22.45±0.64	23.34±0.49	23.05±0.65	22.16±0.53	22.88±0.95	0.213
Proventriculus	0.43±0.03	0.42±0.05	0.45±0.04	0.46±0.03	0.40±0.03	0.41±0.04	0.881
Gizzard	2.38±0.11	2.20±0.09	2.42±0.10	2.49±0.10	2.34±0.06	2.35±0.05	0.397
Liver	2.42±0.10	2.50±0.13	2.66±0.06	2.76±0.11	2.49±0.14	2.56±0.06	0.250
Heart	0.55±0.07	0.50±0.09	0.58±0.06	0.57±0.07	0.49±0.07	0.53±0.08	0.948

**Table 7.** Effect of dietary treatments on intestinal bacterial count (log cfu/g) of broilers at the end of the experiment

Intestinal bacteria	Segment	Group						P-value
		Control	Antibiotic	Probiotics		Acidifiers		
		( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Total aerobic count	S. intestine	8.10±0.74	7.06±0.41	7.46±0.67	6.92±0.30	7.15±0.49	7.87±0.55	0.457
	Caecum	8.88±0.25	8.15±0.29	8.18±0.50	8.31±0.42	8.05±0.25	8.80±0.31	0.204
Lactobacilli	S. intestine	6.19±0.48	5.82±0.60	6.39±0.05	6.53±0.56	6.85±0.54	6.49±0.80	0.632
	Caecum	6.84±0.34	6.30±0.68	6.93±0.34	6.97±0.40	7.04±0.29	7.05±0.40	0.785
Coliforms	S. intestine	5.35±0.66	3.98±0.41	4.54±0.69	3.92±0.28	4.23±0.54	5.10±0.52	0.339
	Caecum	5.76±0.47	5.18±0.29	5.35±0.44	5.06±0.54	4.48±0.44	5.56±0.44	0.174
F. coliform	S. intestine	5.30±0.68	3.89±0.39	3.60±0.29	3.92±0.28	4.11±0.47	4.98±0.58	0.117
	Caecum	5.71±0.51	5.47±0.34	5.02±0.39	4.89±0.55	4.40±0.40	5.31±0.54	0.436
E. coli	S. intestine	3.75±0.09	3.41±0.21	3.60±0.29	3.51±0.13	3.45±0.09	3.48±0.13	0.203
	Caecum	4.06±0.25	3.33±0.22	3.61±0.25	3.44±0.14	3.38±0.08	3.63±0.24	0.156

**Table 8.** Effect of dietary treatments on bacterial count (log cfu/g) in carcass meat of broilers at the end of the experiment

Bacterial type	Group						P-value
	Control	Antibiotic	Probiotics		Acidifiers		
	( - )	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Total aerobic count	5.10 <sup>a</sup> ±0.10	3.10 <sup>b</sup> ±0.10	3.39 <sup>b</sup> ±0.12	3.54 <sup>b</sup> ±0.16	3.46 <sup>b</sup> ±0.09	3.38 <sup>b</sup> ±0.25	0.0001
Coliforms	2.48 <sup>a</sup> ±0.21	2.06 <sup>a</sup> ±0.29	2.55 <sup>a</sup> ±0.28	2.27 <sup>a</sup> ±0.33	2.93 <sup>a</sup> ±0.27	2.96 <sup>a</sup> ±0.27	0.193
F. coliform	2.01 <sup>a</sup> ±0.10	1.51 <sup>a</sup> ±0.03	1.81 <sup>a</sup> ±0.20	1.80 <sup>a</sup> ±0.20	2.35 <sup>a</sup> ±0.22	1.98 <sup>a</sup> ±0.35	0.166
E. coli	1.71 <sup>a</sup> ±0.23	1.32 <sup>a</sup> ±0.21	1.51 <sup>a</sup> ±0.15	1.44 <sup>a</sup> ±0.22	1.39 <sup>a</sup> ±0.20	1.60 <sup>a</sup> ±0.13	0.540
Staphylococcus	3.42 <sup>a</sup> ±0.06	1.39 <sup>c</sup> ±0.21	1.65 <sup>c</sup> ±0.25	1.49 <sup>c</sup> ±0.29	1.43 <sup>c</sup> ±0.30	2.33 <sup>b</sup> ±0.33	0.0001

<sup>a, b, c</sup> Means within the same row with different superscripts are significantly different (P< 0.05).

## DISCUSSION

### Growth performance

Feed additives are considered an indispensable part of feed manufacture and animal nutrition. Substitution of conventional antibiotic growth promoters with alternative feed additives has received great attention in the recent past since the European Union and many countries have banned using antibiotics as growth promoters in poultry nutrition (Reid and Friendship, 2002). Improvement in body weight and weight gain of birds fed diets supplemented with probiotics and lactic acid, especially during the grower period, is thought to be induced by their effects on maintenance of beneficial bacteria population, and improving nutrient digestion (Jin et al., 1997; Adil et al., 2010; Getachew, 2016; Khan and Iqbal, 2016). However, salmo-nil treatment did not affect the body weight or weight gain in this study. The beneficial observations of organic acids are not consistent because the benefits of these acids are related to several variables including the kind of organic acid used, dosage, buffering capacity of dietary ingredients, as well as sanitation level of the production environment (Dibner and Buttin, 2002). In addition, feed palatability may be affected by the sources and inclusion levels of dietary organic acids, and therefore seems to affect the efficacy of these acids (Kim et al., 2015). Moreover, the feed intake was not affected by the feed additives during the starter period, but reduced (about 8 %) in biopellet-s and acidifiers treatments than the control throughout the grower period. However, all the tested feed additives improved the feed conversion efficiency in starter and grower periods. All over the experimental period (0-6 week), the performance indices were similar to that observed during the grower period. The positive effect of probiotics on growth performance in the present study is also reported in other studies (Mountzouris et al., 2007; Samli et al., 2007; Awad et al.,

2009; Pourakbari et al., 2016). Furthermore, the influence of lactic acid on performance is consistent with the findings of other researchers (Runho et al., 1997; Adil et al., 2010; Bhanjat et al., 2010). The improved growth performance by lactic acid is probably due to the beneficial effect of the acid on the intestinal flora. The organic acids may affect the integrity of microbial cell membrane or hinder the nutrient transport and energy metabolism causing the bactericidal effect (Ricke, 2003). Besides, butyric acid has been reported to decrease the colonization of bacteria in the caeca of broiler chicken (Van Immerseel et al., 2004). In addition, the increased feed conversion efficiency in lactic acid group could be due to the enhanced utilization of nutrients resulting in increased body weight gain (Adil et al., 2010). Moreover, the obtained results indicate that the effect of feed additives on performance becomes more pronounced within the grower period, but their effects appear to be cumulative commenced at the starter phase. However, the effect of salmo-nil on the performance data is supported by the findings reported by Paul et al. (2007) who found that the use of the organic acid salts in broiler diets reduced feed intake, but the body weight gain was similar to control birds and thus improved FCR. Based on performance indices, no difference between both types of probiotics was detected, indicating that the mode of action of different probiotics is nearly identical. The same finding was also found between lactic acid producing bacteria (bactocell) and lactic acid per se, suggesting that the effect of lactic acid produced by bacteria is comparable to the chemical one. In addition, lactic acid appears to have more beneficial effect than the organic acid mixture. Moreover, the effect of probiotics and lactic acid on performance seems to be identical to that of antibiotic growth promoters. The same findings were reported in previous studies with probiotics (Bai et al., 2013; Tabidi et al., 2013) and lactic acid (Bhanjat et al., 2010).



Throughout the experiment, the feed additives had no effect on the excreta quality as observed by no change in its moisture content. This finding indicates that these compounds have a potential effect on modulation of intestinal microflora and pathogen inhibition (Mountzouris et al., 2007; Hassan et al., 2010).

### **Blood parameters**

Supplementation of the diets with antibiotic, probiotics and acidifiers did not affect the serum glucose concentration as well as the liver and kidney functions as indicated by no change in the serum levels of SGPT, SGOT, and creatinine. The same findings were reported in previous studies which tested the effect of probiotics (Gheith, 2008; Salim et al., 2011) or organic acids (Hernandez et al., 2006; Abdel Fattah et al., 2008; Adil et al., 2010) on blood metabolites. However, all the additives reduced the cholesterol level in the grower period only. It is reported that the probiotic supplementation significantly reduced the serum cholesterol level of the chickens (Ashayerizadeh et al., 2011; Beski and Al-Sardary, 2015; Pourakbari et al., 2016). The most important way of cholesterol excretion is through synthesis of bile acids from cholesterol in the liver (Wilson et al., 1998). The use of probiotics can degenerate bile salts and de-conjugate production of enzymes by the activity of lactic acid bacteria, as well as reduction of the pH in the intestinal tract can be effective in decreasing the cholesterol concentration. Solvability of non-conjugate bile acids is reduced at a low pH and consequently, they are absorbed less from the intestine and are excreted more in the excreta (Klaver and Van der Meer, 1993). Consequently, the liver, for re-establishment of the hepatic cycle of bile acids, converts more cholesterol into the tissues and therefore its concentration in the blood is reduced (Ros, 2000). Also, the effect of antibiotic and organic acids on serum cholesterol could be attributed to the reduction of the intestinal pH that was observed in our study. Kamal and Ragaa (2014) reported that blood total lipids and cholesterol decreased significantly by organic acids.

### **pH values of intestinal digesta**

The tested feed additives can reduce the pH of small intestine contents. Probiotics were found to modify the intestinal environment by reducing the pH (Kabir, 2009). Moreover, organic acids supplementation has pH diminishing property, although non-significant, in various gastrointestinal segments of the broilers (Abdel-fattah et al., 2008). The reduced pH is helpful for the growth of favourable bacteria by simultaneously hindering the growth of pathogenic bacteria which grow at a relatively

higher pH. Nevertheless, the feed additives did not influence the pH of caecum. It is possible that the effects of organic acids in the distal part of the digestive tract decrease because of the reduction in concentration of acids as a result of absorption and metabolism (Bolton and Dewar, 1964). Thus, it can be assumed that the effect of organic acids in the distal segments of the intestinal tract could be due to the reduced entry of pathogenic bacteria from the upper portions of intestinal tract as a compensatory mechanism but no valid literature concerning such mechanism was found.

### **Carcass characteristics**

In this study, the antibiotic, probiotics and lactic acid showed an insignificant increase in the carcass yield percentage (1 to 3 %) when compared to the control. This may be attributed to the greater live body weight of these birds. Recently, Falaki et al. (2011) reported that probiotic supplementation significantly improved the carcass weight, but without any significant influence on the carcass yield. Moreover, the relative weights of breast and internal organs were not affected by the dietary treatments. The impact of probiotics on the relative weights of tested organs is consistent with that noticed by Awad et al. (2009). Concerning to acidifiers, their effects are supported by the results of other investigations which found that the organic acids did not affect dressing yield and carcass characteristics of broiler chicken (Adil et al., 2010; Kopecký et al., 2012; Attia et al., 2013; Ghasemi et al., 2014).

Based on the results of carcass characteristics, no significant difference between both types of probiotics was recorded. The same observation was found between lactic acid and salmo-nil treatments. Likewise, the effect of lactic acid produced by bacteria tended to be similar to that of lactic acid per se. However, probiotics seem to produce more beneficial effects in carcass yield among the treatments, followed by lactic acid and antibiotic products.

### **Microbiological examination**

The antibiotic, probiotics and acidifiers reduced the count of pathogenic bacteria especially total aerobes, coliforms and *E. coli*. The inhibitory effect of probiotics and acidifiers could be attributed to a decrease in intestinal pH (Fuller, 1989; Boroojeni et al., 2014). Sakata et al. (2003) reported that probiotic bacteria actually increase the production rates of volatile fatty acids and lactic acid. Several mechanisms related to the antagonistic effects of probiotics on various microorganisms include secretion of antimicrobial substances, competitive adherence to the intestinal mucosa, and stimulating the immune system

(Collado et al., 2010). Organic acids can perforate the bacteria cell wall and upset normal cellular functions (Davidson, 2001). The increased count of *Lactobacilli* in probiotics and acidifiers could be attributed to their effect in stimulating the growth of beneficial bacteria and suppressing the pathogenic one (Van Immerseel et al., 2006; Getachew, 2016; Waseem Mirza et al., 2016).

The used feed additives have the ability to improve the keeping quality of the carcass meat through its role in reducing the total aerobes, *Staphylococcus* and *E. coli* counts. Kabir (2009) reported that probiotics improved the meat quality via diminishing *Staphylococcus* and *E. coli* counts in broiler meat. Moreover, organic acids have been observed to have bactericidal effects on pathogenic bacteria (Kim et al., 2015; Khan and Iqbal, 2016). In addition, lactic acid can be used to reduce the bacterial contamination of broiler carcasses (Byrd et al., 2001). The effect of tested additives on the bacterial count of meat could be also attributed to its activity in lowering the count of intestinal pathogens.

## CONCLUSIONS

Probiotics and lactic acid increased the body weight of broilers as the antibiotic growth promoters. Moreover, the tested probiotics and acidifiers provided a better feed conversion which was similar to that produced by antibiotic. Also, their effects on intestinal and meat pathogens were similar to that of antibiotic, but with stimulating effect on beneficial bacteria. No difference between the used kinds of probiotics was observed. Lactic acid alone seems to produce better performance results than the organic acids blend. The natural lactic acid produced by bacteria could have a comparable effect to that of the chemical one. Finally, the obtained results indicate that the probiotics and organic acids are promising alternatives to antibiotics in diets of broilers.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

All authors participated in making the design, performing the experiment, analyses of the data, and writing the paper.

## REFERENCES

- Abdel-Fattah SA, El-Sanhoury MH, El-Mednay NM and Abdel-Azeem F (2008). Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. *International Journal of Poultry Science*, 7: 215–222. DOI: 10.3923/ijps.2008.215.222.
- Adil S, Banday T, Ahmad B, hat G, Saleem Mir M and Rehman M (2010). Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. *Veterinary Medicine International*, Article ID 479485, 7 pages. doi:10.4061/2010/479485.
- Andremont A (2000). Consequences of antibiotic therapy to the intestinal ecosystem. *Annales Françaises d'Anesthésie et de Réanimation* 19: 395–402. doi.org/10.1016/S0750-7658(00)90209-0.
- AOAC (2005). Official Methods of Analysis of AOAC International, 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- APHA (1992). Standard Methods for Examination of Water and Wastewater, 18th Edition. American Public Health Association. Washington DC.
- Ashayerizadeh A, Dabiri N, Mirzadeh KH and Ghorbani MR (2011). Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. *Journal of Cell and Animal Biology*, 5: 152-156. doi: 1A53EB113509.
- Attia YA, Abd El-Hamid AE, Ellakany HF, Bovera F, Al- Harthi MA and Ghazaly SA (2013). Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. *Italian Journal of Animal Science*, 12: 222-230. doi.org/10.4081/ijas.2013.e37.
- Awad WA, Ghareeb K, Abdel-Raheem S and Böhm J (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, 88:49–55. DOI: https://doi.org/10.3382/ps.2008-00244.
- Bai SP, Wu AM, Ding XM, Lei Y, Bai J, Zhang KY and Chio JS (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry Science*, 92:663–670. http://dx.doi.org/10.3382/ps.2012-02813.
- Beski SSM and Al-Sardary SYT (2015). Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. *International Journal of Poultry Science*, 14: 31-36. DOI: 10.3923/ijps.2015.31.36.

- Bhanjat SK, Anjali DC, Shyam SG and Panda AK (2010). Effect of lactic acid and antibiotic on the performance of broiler chickens. *Indian Journal of Animal Nutrition*, 27 (3): 290-293.
- Bolton W and Dewar WA (1964). The digestibility of acetic, propionic and butyric acids by the fowl. *British Poultry Science*, 6: 103-105. <http://dx.doi.org/10.1080/00071666508415562>.
- Borojeni FG, Vahjen W, Mader A, Knorr F, Ruhnke I, Röhe I, Hafeez A, Villodre C, Männer K and Zentek J (2014). The effects of different thermal treatments and organic acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. *Poultry Science*, 93: 1440-1452. DOI: 10.3382/ps.2013-03763.
- Burgat V (1999). Residues of drugs of veterinary use in food. *La Revue Du Praticien*, 41: 985-990. PMID:2063112
- Byrd JA, Hargis BM, Caldwell DJ, Bailey RH, Herron KL, McReynolds JL, Brewer RL, Anderson RC, Bischoff KM, Callaway TR and Kubena LF (2001). Effect of lactic acid administration in the drinking water during pre-slaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. *Poultry Science*, 80:278-283.
- Collado MC, Gueimonde M and Salminen S (2010). Probiotics in adhesion of pathogens: mechanisms of action; in Watson RR, Preedy VR (eds): *Bioactive Foods in Promoting Health*, Chennai, Academic Press, Elsevier 23: 353-370. DOI: 10.1016/B978-0-12-374938-3.00023-2
- Davidson PM (2001). Chemical preservatives and natural antimicrobial compounds. In: Doyle MP, Beuchat LR, Montville TJ, editors. *Food microbiology-fundamentals and frontiers*. 2nd ed. Washington, DC: American society for microbiology, p.593-627. DOI: 10.1128/9781555815912.ch33.
- Denli M, Okan F and Celik K (2003). Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pakistan Journal of Nutrition*, 2: 89-91. doi=pjn.2003.89.91
- Dhawale A (2005). Better eggshell quality with a gut acidifier. *Poultry International*, 44: 18-21. <https://www.researchgate.net/.../286331926>
- Dibner JJ and Buttin P (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *Journal of Applied Poultry Research*, 11:453-463. <https://doi.org/10.1093/japr/11.4.453>
- Falaki M, Shams Shargh M, Dastar B and Zerehdaran S (2011). Effect of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens. *Journal of Animal and Veterinary Advances*, 10:378-384. DOI: 10.3923/javaa.2010.2390.2395
- Fuller R (1989). Probiotic in man and animal. *Journal of Applied Bacteriology*, 66: 365-378. DOI: 10.1111/j.1365-2672.1989.tb05105.x.
- Getachew T (2016). A review on effects of probiotic supplementation in poultry performance and cholesterol levels of egg and meat. *Journal of World Poultry Research*, 6(1): 31-36. PII: S2322455X1600006-6.
- Ghasemi HA, Akhavan-Salamat H, Hajkhodadadi I and Khaltabadi-Farahani AH (2014). Effects of dietary organic acid blend supplementation on performance, intestinal morphology and antibody-mediated immunity in broiler chickens. *Acta Advances in Agricultural Sciences*, 2: 64-74. doi.AAAS-2014-50344376.
- Gheith IM (2008). Clinicopathological studies on the effect of probiotic in broilers. M.V.Sc. Thesis, Fac. Vet. Med., Benha University. [www.bvmj.bu.edu.eg/issues/22-2/5](http://www.bvmj.bu.edu.eg/issues/22-2/5).
- HassanHMA, MohamedMA, YoussefAW and HassanER (2010). Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. *Asian-Australian Journal of Animal Science*, 23: 1348 - 1353. DOI: 10.5713/ajas.2010.10085.
- Hernandez F, Garcia V, Madrid J, Orengo J, Catala P and Megias MD (2006). Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. *British Poultry Science*, 47: 50-56. DOI: 10.1080/00071660500475574
- Hossein G, Farshid K and Mostafa F (2017). Effect of using protexin probiotic and ephedra funereal powder supplementation on performance and some carcass traits on broiler chicks. *Scholarly Journal of Agricultural Science*, 7(1): 6-10. Available online at <http://www.scholarly-journals.com/SJAS>.
- ICMSF (1978). International commission on microbiological specifications for foods. *Microorganisms in foods*. 2. Sampling for microbiological analysis: Principles and specific

- applications. University of Toronto Press, Toronto.
- Jin LZ, Ho YW, Abdullah N and Jalaludin S (1997). Probiotics in poultry: Modes of action. *World's Poultry Science Journal*, 53: 352–368. DOI: 10.1079/WPS19970028.
- Kabir SML, Rahman MM, Rahman MB, Rahman MM and Ahmed SU (2004). The dynamics of probiotics on growth performance and immune response in broilers. *International Journal of Poultry Science*, 3: 361–364. DOI: 10.3923/ijps.2004.361.364
- Kabir SML (2009). The role of probiotics in the poultry industry. *International Journal of Molecular Science*, 10: 3531–3546. doi: 10.3390/ijms10083531.
- Kamal AM and Ragaa NM (2014). Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken. *Nature and Science*, 12: 38–45. doi:10.7537/marsnsj120214.06.
- Katoch S, Thakur S and Rajput R (2017). Effect of probiotic supplementation in broiler birds offered feed formulated with lower protein densities. *International Journal of Livestock Research*, 7(2):90–102. doi:10.5455/ijlr.20170205013927.
- Khan SH and Iqbal J (2016). Recent advances in the role of organic acids in poultry nutrition. *Journal of Applied Animal Research*, 44 (1): 359–369. DOI: 10.1080/09712119.2015.1079527.
- Kim JW, Kim JH and Kil DY (2015). Dietary organic acids for broiler chickens: a review. *Revista Colombiana de Ciencias Pecuarias*, 28: 109–123. doi: 10.17533/udea.rccp.v28n2a01.
- Kirchgessner M and Roth FX (1988). Ergotrope effekte durch organische sauren in der fekelauzucht und schweinmast. *Übersichten zur Tierernährung*, 16: 93–108. ISSN 0303-6340.
- Klaver FAM and van der Meer R (1993). The assumed assimilation of cholesterol by *Lactobacilli* and *Bifidobacterium bifidum* is due to their bile salt deconjugating activity. *Applied Environmental Microbiology*, 59: 1120–1124. Doi:0099-2240/93/041120-05\$02.00
- Kopecký J, Hrnčár C and Weis J (2012). Effect of organic acids supplement on performance of broiler chickens. *Animal Sciences and Biotechnologies*, 45: 51–54. ISSN online 2344 - 4576
- La Ragione RM and Woodward MJ (2003). Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype Enteritidis and *Clostridium perfringens* in young chickens. *Veterinary Microbiology*, 94:245–256. DOI: 10.1016/S0378-1135(03)00077-4.
- Langhout P (2000). New additives for broiler chickens. *World Poultry*, 16: 22–27. doi:20001416551.
- Mountzouris KC, Tsistsikos P, Kalamara E, Nitsch S, Schatzmayr G and Fegeros K (2007). Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science*, 86: 309–317. <https://www.ncbi.nlm.nih.gov/pubmed/17234844>.
- Nahanshon SN, Nakaue HS and Mirosh LW (1993). Effects of direct fed microbials on nutrient retention and parameters of Single White Leghorn pullets. *Poultry Science*, 72: (Suppl. 2), 87. (Abstract)
- National Research Council (1994). Nutrient requirements of poultry. 9th revised edition, National Academy Press. Washington, D.C.
- Nawaz H, Irshad MA, Ali M and Haq A (2016). Effect of probiotics on growth performance, nutrient digestibility and carcass characteristics in broilers. *The Journal of Animal & Plant Sciences*, 26(3): 599–604.
- Patterson JA and Burkholder KM (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science*, 82: 627–631. DOI: <https://doi.org/10.1093/ps/82.4.627>
- Paul SK, Halder G, Mondal MK and Samanta G (2007). Effect of organic acid salt on the performance and gut health of broiler chicken. *Japanese Poultry Science*, 44: 389–395. <http://doi.org/10.2141/jpsa.44.389>
- Pourakbari M, Seidavi A, Asadpour L and Martínez A (2016). Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. *Anais da Academia Brasileira de Ciências*, 88(2): 1011–1021. <http://dx.doi.org/10.1590/0001-3765201620150071>.
- Reid G and Friendship R (2002). Alternatives to antibiotic use: Probiotics for the gut. *Animal Biotechnology*, 13: 97–112. <http://dx.doi.org/10.1081/ABIO-120005773>.
- Ricke SC (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science*, 82: 632–639. DOI: <https://doi.org/10.1093/ps/82.4.632>.

- Ros E (2000). Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. *Atherosclerosis*, 151: 357-379. [https://doi.org/10.1016/S0021-9150\(00\)00456-1](https://doi.org/10.1016/S0021-9150(00)00456-1).
- Runho RC, Sakomura N K, Kuana S, Banzatto D, Junqueira OM and Stringhini JH (1997). Use of an organic acid (fumaric acid) in broiler rations. *Revista Brasileira de Zootecnia*, 26: 1183-1191. <http://hdl.handle.net/11449/35218>.
- Sakata T, Kojima T, Fujieda M, Takahashi M and Michibata M (2003). Influences of probiotic bacteria on organic acid production by pig caecal bacteria *in vitro*. *Proceedings of the Nutrition Society*, 62: 73-80. DOI:10.1079/PNS2002211.
- Salim HA, Abd-Allah OA and Fararh KM (2011). Effect of feeding probiotic on hematological, biochemical properties and immune response in broiler. *Benha Veterinary Medical Journal*, 22: 35-43.
- Samli HE, Senkoylu N, Koc F, Kanter M and Agha A (2007). Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and microbiota. *Archives of Animal Nutrition*, 61: 42-49. <http://dx.doi.org/10.1080/17450390601106655>.
- Sorum H and Sunde M (2001). Resistance to antibiotics in the normal flora of animals. *Veterinary Research*, 32: 227-241. <https://doi.org/10.1051/vetres:2001121>.
- Tabidi MH, Mukhtar AM and Hassan IM (2013). Effects of probiotic and antibiotic on performance and growth attributes of broiler chicks. *Global Journal of Medicinal Plant Research*, 1(1):136-142.
- Van Immerseel F, De Buck J, De Smet I, Pasmans F, Haesebrouck F and Ducatelle R (2004). Interactions of butyric acid- and acetic acid-treated *Salmonella* with chicken primary cecal epithelial cells *in vitro*. *Avian Diseases*, 48: 384-391. doi: <http://dx.doi.org/10.1637/7094>.
- Van Immerseel F, Russell J, Flythe M, Gantois I, Timmermont L, Pasmans F, Haesebrouck F and Ducatelle R (2006). The use of organic acids to combat *Salmonella* in poultry: A mechanistic explanation of the efficacy. *Avian Pathology*, 35: 182-188. <http://dx.doi.org/10.1080/03079450600711045>
- Waseem Mirza M, Rehman ZU and Mukhtar N (2016). Use of organic acids as potential feed additives in poultry production. *Journal of World's Poultry Research*, 6(3): 105-116. PII:S2322455X1600015-6.
- Wilson TA, Nicolosi RJ, Rogers EJ, Sacchiero R and Goldberg DJ (1998). Studies of cholesterol and bile acid metabolism, and early atherogenesis in hamsters fed GT16-239, a novel bile acid sequestrant (BAS). *Atherosclerosis*, 140: 315-324. [https://doi.org/10.1016/S0021-9150\(98\)00135-X](https://doi.org/10.1016/S0021-9150(98)00135-X).
- Youssef IMI, Beineke A, Rohn K and Kamphues J (2012). Influences of increased levels of biotin, zinc or mannan-oligosaccharides in the diet on foot pad dermatitis in growing turkeys housed on dry and wet litter. *Journal of Animal Physiology and Animal Nutrition*, 96 (5): 747-761. DOI:10.1111/j.1439-0396.2010.01115.x



# Understanding of Social and Mating Behaviour of Ostrich (*Struthio camelus*)

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## ABSTRACT

The ostrich is the largest wild ratite bird. The head of ostrich is 1.8-2.75m above ground due to large legs. The ostrich is the largest vertebrate and achieves a speed of 60-65km/h. There are four extinct subspecies and limited to Africa. The preferred habitat in nature is the open area, small grass corners and open desert. They choose more open woodland and avoid areas of dense woodland and tall grass. In natural environment, ostrich is gregarious and lives in groups. This small crowd are led mature sire or dam. Walking, chasing and kantling are exhibited to protect the territories by males. Off springs are protected by adults from predator by mock injury. Other behaviours are yawning, stretching and thermoregulation. Frequency of mating is low in captivity. Mostly male-female ratio is 1:2 (Male: Female) kept in experiment and ostriches are selective in case of their mates and they might direct their courtship displays at humans rather than their mates, due to the presence of humans around in captivity. The breeding behaviour of ostriches is improved due to external application of L-carnitine-magnesium supplement.

**Keywords:** Ostrich, Mating, Behaviour, Courtship, Breeding

## INTRODUCTION

Ostrich is the largest Ratite (flightless birds without keel bone) bird, 2.75m in height and 150kg in mass and its feathers are fluffy and symmetrical (Brown et al., 1982; Cramp et al., 1997; Deeming and Angel, 1996). The adult male bird has a grey colored neck with black and white wing primaries and tail feathers. The female has white to light grey wing primaries and tail feathers and body have color pattern dull brown to grey. Growing ostrich have mottled brown, yellow, cream color and orange with black quills on back while juvenile birds resemble the females (Brown et al., 1982 and Cramp et al., 1987). The head is 1.8-2.75m above the ground due to the combination of large legs. The eyes are 50mm in diameter. Ostrich is the largest vertebrate and has the ability to position his head to produce an image from in front of and below the eye (Brown et al., 1982). The large blind spots on above and behind the head are considered to shade the eye (Martin and Katzir, 1995). Ostriches spent

most of their time for eating standing and walking (Menon et al., 2014). Ostrich only runs in case of any danger and achieves a speed of 60-70km/h (Cramp et al., 1977). They can run and walk at a rate of 15-25 km per day (Patodkar et al., 2009). Ostrich is a digitigrades with two toes. Though ostrich can't fly, the presence of air sacs, pneumatized bones, strong pygostyle and presence of some wing bones give evidence that ostrich is evolved from flying ancestor (Cramp et al., 1977; Burning, 1991). Ostrich behaviour can study with three established methods like experimental behavior, comparative behavior and observational behavior (Jackson, 2009; Ahmed and Salih, 2012).

## Habitats

The preferred habitat is open, short grass plains and semi-arid desert. They prefer more open woodland and avoid areas of dense woodland and tall grass. The bird tends to live in dry grassland and lowland areas (Brown et al., 1982; Unattributed, 2012). Currently, it was indicated



that the high food availability was more effective on group size of animals and place of habitat in southeastern of Brazil (De Azevedo et al., 2010). The ostriches of Southern Africa can be found in desert grassland, Karoo shrubland, coastal fynbos and semi-arid savanna (Dean et al., 1994). Coastal fynbos is unique to South Africa due to the presence of small shrub bushes of the Protea and Erica families (Chambers and Odendaal, 1996). Bird densities are approximately one per 5-20 km<sup>2</sup> but in protected areas their density is 0.8 bird km<sup>2</sup> (Brown et al., 1982).

### **Behaviour of ostrich**

One feature of behaviour is that the ostrich bury their head in sand and this behaviour has no scientific background (Pocock, 1955). Instead there is a coincidence of ostrich's head to the horizon when lying or feeding (Sauer and Sauer, 1966a). Frequency of behaviour changes in winter and summers seasons reported by Ross and Deeming (1998) and McKeegan and Deeming (1997).

### **Social behaviour**

Other than breeding behaviour, ostriches live in groups especially for water and food (Burger and Gochfeld, 1988; Bertram, 1992). The adult have no any specialized pattern for social behaviour and seen to be in herd of 10-20 ostriches (Sauer and Sauer, 1966a; Musi et al., 2008). There appears to be a social chain of command within these groups, headed by a mature sire or dam (Sauer and Sauer, 1966b; Unattributed, 2012). Gender pairing was observed in 1:1 to 1:4 ratios (Musi et al., 2008). It is reported that 80% of ostriches were seen with a single male and 20% engage in a relationship with more than one male ostrich in breeding season (Bertram, 1992; Blach et al., 2000). In natural habitats the ostriches live in a mixed community. They encounter the wide variety of other species of animals. Sauer (1970) investigated in Namibia on the interspecific behaviour of ostriches by knowing the results of contact of ostrich with mammals and birds. About 75% of the interspecific behaviours are largely neutral by avoiding and tolerating other species and avoiding close contact with other animals as well. Due to this shyness ostrich became a difficult species to observe in natural conditions (Bertram, 1992). Similarly, about 16% of encounters occurred due to the opposite behaviour against other species (Sauer, 1970).

### **Courtship and breeding**

Sexual behaviour of adult ostriches in the social groups was studied by Sauer and Sauer (1966 a, b). It was observed that females showed pre-nuptial courtship by posturing in front of the potential mates. Females

showed violent behaviour against other females and young yearlings. Young yearling showed submissive behaviour by lowering head and neck in S-shape. The development of typical red flush beak, thigh, neck and shin skin in males and courtship behaviour developed later in males in contrast to females and it was also a slow process. Males exhibited dominant behaviour in mix-groups by posturing usually with tail held erect and violent against other members. The straight phallus is also distended from cloaca and showed to other birds. Males exhibited territories by making nest scrapes (Sauer and Sauer, 1966b). The territories vary between 11 to 19 km<sup>2</sup> though immature males hold smaller territories in Kenya (Bertram, 1992). There is comparatively little overlap between territories and this same area can be utilized by males in consecutive years. Walking, chasing and kantling are exhibited to protect the territories by males. In contrast females covered a mean range of at least 25 km<sup>2</sup> and they go through the territories of males, however, few males were completely avoided by some females. Courtship behaviour leads to copulation (Sauer and Sauer, 1966b) and booming sound of males help to draw the attention of females toward courtship behaviour. The both gender show synchronized feeding behaviour and this can be easily disturbed by off-grazing the animals. The next step is ritualized feeding by both birds at selected nest site. The male then walks with steps and fixed their neck by moving forward and backward and swing their wings. The male then drops to kantle the female on ground, sitting on its haunches with its wings held forward holding its neck over her back, at regular intervals moving both its head and neck from side to side with its head hitting her back at the completion of each sideways stroke. The female displays her precopulatory behaviour by fluttering her wings and holding them in forward position and lowering her head with support of her beak. This ends with the faeces of ostrich to the ground with her elevated tail and neck forward. The sire mounts on the female. Male stamps its feet various times on ground just earlier to mounting. Mounting involves the male sitting with a leg on each side and to the right side of the female. Before intromission repeated thrust of phallus are often required. During copulation the male performs a kantle (Male specific dance behaviour) show to culmination with its head being brought forward and a deep harsh grunt emitted. The female usually remains expressionless during the 30-60 s of mating though she may hold her head forward and clap her beak. There is a little post-copulation behaviour (Sauer, 1972). Peculiar sexual behaviour has been reported for large groups of male ostriches in Namibia (Sauer, 1972). Typical courtship behaviours included kantling

performed by males to males who did not respond. Sauer (1972) interpreted this as a way of releasing sexual tension prior to breeding or as a method of suppressing violent behaviour associated with a prolonged period of wet weather. Whether this interpretation is correct, it is not clear because kantling is a violent behaviour in male–male confrontations over terrain (Bertram, 1992). Since the behaviour was pragmatic in large groups of males within which it is occasionally difficult to distinguish birds by age, it is possible that the behaviour was being displayed by young, inexperienced males. The ostrich has a mutual nesting system which is widely described by Bertram (1992) and only momentarily reviewed here. Each territorial male digs a number of nest scrapes which he shows to any female which come into its territory. A major female pairs with the male and lays most of her eggs in the nest site she chooses in each territory. Other minor females in addition, visit the territory and may lay an egg within a previously established nest. These females may be ‘major’ females in another male’s territory. The typical number of minor females laying in a nest is three (ranges from one to five). Each ‘major’ hen generally contributes about 11 eggs (in the range of 9–14) to her laying nest and 26 eggs were laid in a clutch. This breeding design is reported for birds all the way through the natural range of the ostrich (Bertram, 1992; Musi et al., 20008; Patodkar et al., 2009) and consequently considered being typical of the species. In late afternoon and early evening eggs were laid (Sauer and Sauer, 1966b). Bertram (1992) reported that clutch build up over a period of up to 30 days. During the clutch time the nest is attended by both sexes (Sauer and Sauer, 1966b). Incubation is carried out by both males and females, with the male bird sitting during the night (Bertram, 1992). At the first sign of danger the birds depend on camouflage to hide them from predators, even though they perform diversion displays or attack potential predators if required (Sauer and Sauer, 1966b; Bertram, 1992). One strange feature of the ostrich breeding system is once the ‘major’ hen starts to incubate she rearranges the eggs and destroys several from the nest until approximately 19–20 eggs remain (Bertram, 1979, 1992). These eggs lie in a ring around the incubating bird and do not develop. Conversely to the view of Sauer and Sauer (1966b), the major hen actively destroys eggs laid in the nest by minor hens and retains her own. How she recognizes her own eggs is still not clear (Bertram, 1979).

### **Behaviour of offspring**

Incubation period of ostrich s 42–44 days, during which time the chicks remain brooded by an adult although they start to utilize small stones during periods of

activity (Sauer and Sauer, 1966b). Chicks are difficult to track in grassland, once they leave the nest but parents of the chicks brood them as protection against the rudiments and predators (Bertram, 1992). Adults will pretend a ‘mock injury’ to divert a potential predator from the chicks (Brown et al., 1982; Bertram, 1992). Families of chicks are pooled into creches, which may number up to 300 birds and are overseen by a single pair of adult birds (Hurxthal, 1979). When groups of chicks gather there is a dynamic behavioural competition between the guardians of each group over which adults will take charge of the enlarged creche. Generally younger chicks are accepted into groups of older chicks, but not vice versa (Brown et al., 1982). By the time the chicks are a year old they have been neglected by the adults and spend their time in a dense peer group (Bertram, 1992).

### **Other behaviours**

The behaviour of ostriches diverges according to the age and day period (Amado et al., 2011). Ostriches carry out a variety of maintenance activities including yawning and stretching (Sauer and Sauer, 1967). Yawning habitually precedes sleep, simultaneously stretching, takes place after waking. Ostriches are skilled at behavioural thermoregulation. Ostriches frequently preen feathers with their beaks (grooming behaviour) and they do this while walking, sitting, standing, or even in rainy days (Menon et al., 2014).

Thermoregulation is mechanisms by which warm-blooded birds try to constant their core temperature. For desert-adapted birds like the ostrich, the main problem is to avoid overheating. During hot weather they release heat by panting and by seeking shade (Louw, 1972).

### **Captive environment**

#### **Adults Behaviour**

In a study carried out in South Africa, the behaviour of ostriches managed on ranches of natural vegetation surrounded by a boundary enclosure has been investigated and compared with sheep under the same circumstances. It was observed that 43% of the daylight hours were spent in walking, fighting and running by ostriches while sheep maintained at same conditions spend 19% of their time in performing the same activities (Milton and dean, 1995). Male ostriches perambulation boundary fences and even when provided with additional rations they forage from the veld. At stocking densities of 1–10 ha/bird/year, ostriches destroy the veld through path formation and trampling of vegetation (Milton and Dean, 1995; Unattributed, 2012). A simple time–activity plan over the period of daylight hours was recorded for a group of 120

adult ostriches (40:80 males: females) maintained in a 30 ha enclosure in Israel (Sambraus, 1994a). Ostrich 63% time spent on walking and standing. Sitting and lying averaged 18.7% and low in morning but increased in afternoon. Eating and drinking combined averaged 18.3% with a peak during the morning after the concentrate ration had been delivered to ostriches (Sambraus, 1994a).

**Table 1.** Behaviour pattern in captivity as reported by Berendsen (1995)

Behaviour	Time spent
Drinking	1.1 %
Drinking	26 %
Walking / Running	15 %
Resting	2 7%
Standing	18 %
Preening	0.06 %
Fence pecking	3 %
Courtship	1%
Aggressive behaviour	1.9%

More complete time–activity plans are accessible for breeding ostriches maintained during summer season (Ross and Deeming, 1998) and winter (Deeming, 1998a). Six behaviours dominated in behaviour are standing, pacing, walking, sitting, feeding (concentrate ration) and foraging (pasture). During summer months, trios and pairs shows the major differences in behaviours (McKeegan and Deeming, 1997). Females gave less speeded and walked as compare to females, while the female has spent more time on foraging and feeding than males (McKeegan and Deeming, 1997). Eleven males and 22 females maintained in two larger groups, showed largely comparable results, though in males the frequency of pacing was greatly abridged, with an increase in standing and sitting (McKeegan and Deeming, 1997). These differences were accredited to territoriality by males (McKeegan and Deeming, 1997; Berendsen, 1995). Little behaviour was affected by the time of day though the frequency of feeding increased, and the frequency of foraging decreased, in the period instantaneously after food delivery. In all group sizes the incidence of sitting behaviour was the highest just before sunset (McKeegan and Deeming, 1997). Sitting was observed at a more recurrent rate than in Britain, and females sat for more time than males. Standing was alike for both sexes whereas the frequency of pacing was over twofold as much in males than females. The frequency of foraging in females was nearly three times higher than in males. The frequency of other behaviours was equivalent in the two

sexes. Feeding time was higher in morning but foraging time is higher after afternoon. This was accredited to the management for providing food during the morning and the ostriches' supplementation diet with grazing in the afternoon (Deeming, 1998a). The effects of particular climatic conditions on ostrich behaviour have been investigated during spring and winter months in Britain (Deeming, 1997b, 1998b). During periods of rain, energetic behaviours such as pacing and foraging were decreased but feeding on concentrate ration was not considerably affected. In the spring, adult males and females spent over 50% of their time sitting during rain whereas only 10–20% during dry weather (Deeming, 1997b). The increase in sitting behaviour was due to the rainfall rather than temperature though the birds generally chose to sit in the rain instead of shelter (Deeming, 1997b). Such behaviour has been pragmatic in ostriches in Germany (Berendsen, 1995). It was observed that in dry conditions (during spring months), feeding and foraging takes only 25–30% of the pragmatic behaviours. During both spring and winter months, when the birds were not yet breeding, there were no sex differences in behaviour (Deeming, 1997b, 1998b). The effects of snow on behaviour have not been reported, but during icy conditions ostriches emerge to walk more vigilantly (Reiner et al., 1996).

### Social behaviour in captivity

In Namibia, Sambraus (1995) studied the social structure of three groups of nine to ten adult ostriches of mixed sex (Male: Female; 4:6). In each of the three groups the alpha and beta positions in the social pecking order were held by older male birds even though being male did not assure a high position in the social structure. Females usually take lower positions in the ranking order. Little is known about agonistic behaviours in farming ostriches. Aggression against females by juvenile males has been described by Stewart (1994) and violence by adult birds is normally directed towards younger chicks (Bolwig, 1973).

### Courtship behaviour in captivity

The pattern of courtship behaviour of captive ostriches is closely related to wild behaviour (Musi et al., 2008; Bubier et al., 1998). Incidence of mating by ostriches in captivity in Britain is low, with only 20 attempts at copulation being recorded in 99 hours of observations (McKeegan and Deeming, 1997). The courtship and coupling trend was higher after sunrise (Sambraus, 1994b). The mated female ostriches store spermatozoa in the oviduct tubules and release them

during fertile period which contained a maximum of six fertilized eggs (Patodkar et al., 2009).

In a study which compared rates in the presence and absence of humans, high rates of copulation were reported in ostriches in the presence of a human standing and adjoining to the enclosure fence (Bubier et al., 1998). Breeding enclosures can be small and hold a pair or trio of birds in the farming conditions. In Europe some enclosures hold large groups of ostriches in a 1:2 (Male: Female) ratio. Large breeding camps in South Africa and Israel are established with 150–200 birds (1:2; Male: Female). In the present systems, the birds were left free to select their own mates (Hicks-Alldredge, 1996). Deeming and Angel (1996) showed that the pairs or group of three ostriches in Britain are more productive than larger groups. Behavioural troubles with view to the choice and number of mates have been recommended by numerous authors to cause a problem in commercial production (Stewart, 1994; Hicks-Alldredge, 1996; Deeming, 1997a), although few studies back up these assertions. In Britain, ostriches in pens containing one male with two females had low egg production and fertility, both of which drastically enhanced when one of the females in each pen died (Deeming and Angel, 1996). In Israel, farming ostriches displayed abnormal courtship behaviours, with females both exhibiting and trying to mount other females (Sambraus, 1994b). Kantle displaying by a female to another female in a neighboring enclosure has been observed in Britain (Deeming and Angel, 1996). The significance of these unreliable reports is not clear, but it is clear that mate selection in captive ostriches needs further investigation.

The courtship reaction of ostriches to humans can be very noticeable. In a study by Bubier et al. (1998), birds were observed for 10 min periods from remote locations where the birds could not see the human, and then for 10 min periods with the human standing after that fence. Male kantling and wing swinging and female pleading for did not occur during data sessions when the ostriches were under observation from a distance and were only observed when the human was next to the barrier (Unattributed, 2009). Adult birds were also observed for 10 min periods before and after disclosure to a human next to the perimeter. No considerable differences in the courtship behaviours in these periods were observed telling that contact with humans did not excite courtship behaviour afterward, although the frequency of copulation whilst the human was present was higher than observed before or after contact. This study suggests that ostriches reared on farms may be interested in humans. If this is the case, this would cause problems at maturity when adult ostriches

might then direct their courtship displays at humans rather than at mates, as observed in this study. Lambrechts and Cloete (1998), who split pairs of adult ostriches into two groups of ten based on whether they had produced less than 30 eggs, or more than 60 eggs, in the preceding breeding season. Observations over a period of 6 months during the breeding season revealed that consumption of concentrate feed by males was significantly higher in the low productivity group, whereas the frequency of mating in this group was considerably lower. The behaviour of breeding adult ostriches is reported to be affected by external application of L-carnitine–magnesium supplement (Lambrechts et al., 1998). When it is compared with birds in the control group, incidence of sitting was lower in both sexes in the treatment group. The occurrence of mating in males and the frequency of foraging in females were also notably increased by variety of food. Although the treatment was premeditated to affect the energy metabolism of the birds, the full importance of these results needs further exploration.

## CONCLUSION

Authors concluded that the behaviour of ostrich is difficult to observe during natural conditions because ostrich is a shy animal. Ostrich exhibits territorial behaviour and consumes fresh natural vegetation during natural environment. Their offspring's were protected by them from predators in natural condition while they show aggression toward younger chicks in captivity. They exhibit thermoregulation behaviour in natural environment. During captivity ostrich can also eat concentrate ration. Incidence of mating was low in captivity as compared to natural environment. In short, Ostriches experience stress in captivity which increases their feeding requirements. Paradoxically the same stress makes them less likely to feed. Hence natural conditions appear best to elicit a normal behaviour. However, further studies are needed to explore its social and mating behaviours parameters.

## Consent to publish

All authors have agreement for publishing this article and have no objection.

## Authors' contribution

All authors have equal contribution.

## Competing interests

The authors declare that they have no competing interests.



## REFERENCES

- Ahmed FAM and Salih RRM (2012). Some behavioral traits of red neck ostrich under captive conditions. *Journal Veterinary Science and Technology*, 3(2):1-3. DOI: <http://dx.doi.org/10.4172/2157-7579.1000117>
- Amado MF, Xavier DB, Boere V, Torres-Pereira C, McManus C and Bernal FEM (2011). Behaviour of captive Ostrich chicks from 10 days to 5 months of age. *Revista Brasileira de Zootecnia*, 40(7): 1613-1618. DOI: <http://dx.doi.org/10.1590/S1516-35982011000700029>
- Berendsen KD (1995). *Ostrich Farm Management*. Landwirtschaftsverlag GmbH, Münster-Hiltrup, Germany, pp. 25–32.
- Bertram BCR (1979). Ostriches recognize their own eggs and discard others. *Nature*, 279:233–234.
- Bertram BCR (1992) *The Ostrich Communal Nesting System*. Princeton University Press, Princeton, New Jersey.
- Blach Barrett CD and Martin GB (2000). Social Mating System and Sexual Behaviour in Captive Emus *Dromaius novaehollandiae*. *Emu*, 100 (3): 161-168. DOI: <https://doi.org/10.1071/MU9902>
- Bolwig N (1973). Agonistic and sexual behaviour of the African ostrich (*Struthio camelus*).
- Brown LH, Urban EK and Newman K (1982) *The birds of Africa*, Vol. I. Academic Press, London, pp. 32-37.
- Bruning D (1991). *The Cambridge Encyclopedia of Ornithology*. Cambridge University Press, Cambridge, p: 88.
- Bubier NE, Paxton CGM, Bowers Pand and Deeming DC (1998). Courtship behaviour of farmed ostriches in relations to humans. *British Poultry Science*, 39:477–481.
- Burger J and Gochfeld M (1988). Effect of group size and sex on vigilance in ostriches (*Struthio camelus*): antipredator strategy or mate competition, *Ostrich*, 59:14–20.
- Chambers R and Odendaal F (1996). *Aspects of Life. A Natural History of Southern Africa*. Struik Publishers, Cape Town. Condor, 75:100–105.
- Cramp S, Simmons KEL, Ferguson LIJ, Gillmor HPAD, Hudson R, Nicholson EM, Ogilvie MA, Olney PJS, Voous KH and Wattel J (1997). *Handbook of the Birds of Europe, the Middle East and North Africa. The Birds of the Western Palearctic*, Vol. 1, Ostrich to Ducks. Oxford University Press, Oxford, pp. 37–41.
- Dean WRJ, Milton SJ, Siegfried WR and Jarvis MJF (1994). *Wildlife Ranching: a Celebration of Diversity*. Promedia, Pretoria, pp. 8-16.
- De Azevedo CS, Ferraz JB, Tinoco HP, Young RJ and Rodrigues M (2010). Time–activity budget of greater rheas (*Rhea americana*, Aves) on a human-disturbed area: the role of habitat, time of the day, season and group size. *Acta Ethologica*, 13: 109–117. DOI: 10.1007/s10211-010-0080-7
- Deeming DC (1997a). *Ratite Egg Incubation – a Practical Guide*. Ratite Conference, Buckinghamshire, UK.
- Deeming DC (1997b). Effect of climatic conditions on the behaviour of adult breeding ostriches (*Struthio camelus*) in Britain. *Animal Welfare*, 6:349–356.
- Deeming DC (1998a). Time–activity budgets of adult ostriches (*Struthio camelus*) in a farming environment during the winter. *Applied Animal Behavioural Science*, 59:363–371.
- Deeming DC (1998b). Effect of winter climatic conditions on the behaviour of adult ostriches (*Struthio camelus*) on a farm in Britain. *Animal Welfare*, 7:307–315.
- Deeming DC and Angel CR (1996). Introduction to the ratites and farming operations around the world. Improving our Understanding of Ratites in a Farming Environment. Ratite Conference, Oxfordshire, UK, pp.1–4.
- Deeming DC and Dick ACK (1995). Ingestion of metal objects by ostriches (*Struthio camelus*). *Veterinary Record*, 137:99–100.
- Drenowatz C, Sales JD, Sarasqueta DV and Weilbrenner A (1995). History and geography. *The Ratite Encyclopedia*. Ratite Records, San Antonio, Texas, pp. 3-29.
- Hicks-Allredge KD (1996). *Ratite Management, Medicine and Health*. Krieger Publishing Co., Malabar, Florida, pp. 047–57.
- Holtzhausen A and Kotzé M (1990). *The Ostrich*. C.P. Nel Museum, Oudtshoorn, South Africa.
- Hurxthal LM (1979). Breeding behaviour of the ostrich *Struthio camelus massaicus* Neumann in Nairobi Park. PhD thesis, Nairobi University, Kenya.
- Jackson HD (2009). Comparative behaviour of two juvenile nightjars in captivity 80(1): 59-61. DOI: <http://dx.doi.org/10.2989/OSTRICH.2009.80.1.9.767>
- Lambrechts H and Cloete SWP (1998). Activity budgets of adult breeding ostriches classified according to annual egg production of the previous breeding season. *Animal Production in Harmony with the Environment*. Proceedings of the 36th National Congress of the South African Society of Animal Science, April 1998, University of Stellenbosch, pp. 93–94.
- Lambrechts H, Cloete SWP and Davis HJ (1998). Influence of L-carnitine-magnesium supplement on the behaviour of adult breeding ostriches. Ratites in a Competitive World. Proceedings of the 2nd

- International Ratite Congress, September Oudtshoorn, South Africa, pp.170–172.
- Larson G (1989). The Far Side Gallery 2. Futura, London.
- Louw GN (1972). The role of advective fog in the water economy of certain Namib Desert animals. Symposium of the Zoological Society London 31:297–314.
- Martin GR and Katzir G (1995). Visual fields in ostriches. *Nature* 374:19–20.
- McKeegan DEF and Deeming DC (1997). Effects of gender and group size on the time -activity budgets of adult breeding ostriches (*Struthio camelus*) in a farming environment. *Applied Animal Behavior Science*, 51:159-177.
- Menon DG, Bennett DC and Cheng KM (2014). Understanding the Behavior of Domestic Emus: A Means to Improve Their Management and Welfare—Major Behaviors and Activity Time Budgets of Adult Emus. *Journal of Animals*, vol. 2014: 8 pages, Article ID 938327. DOI:10.1155/2014/938327
- Milton S and Dean R (1995). Gizzard stones and food selection by free-range ostrich implications for management. *East Coast Producers Association* January/February, 4–7.
- Milton SJ, Dean WRJ and Siegfried WR (1994). Food selection by ostrich in southern Africa. *Journal of Wildlife Management*, 58:234–248.
- Musi EZ, Binta MG and Lumba NJ (2008). Behaviour of wild ostriches (*Struthio camelus*) at Mokolodi Nature reserve, Gaborone, Botswana. *Research Journal of Poultry Science*, 2:1-4.
- Patodkar VR, Rahane SD, Shejal MA and Belhekar DR (2009). Behavior of Emu bird (*Dromaius novaehollandiae*). *Veterinary World*, 2(11): 439-440.
- Pocock A (1955). The ostrich hiding its head – a myth. *African Wildlife*, 9:234–236.
- Reiner G, Seitz K and Dzapo V (1996). A survey of farming environment and ostrich behaviour in Germany. *Improving our Understanding of Ratites in a Farming Environment*. Ratite Conference, Oxfordshire, UK, p: 23–24.
- Ross EJ and Deeming DC (1998). Feeding and vigilance behaviour of adult ostriches breeding in a farmed environment in Britain. *British Poultry Science*, 39:173–177.
- Samraus HH (1994a). The circadian rhythm in the behaviour of ostriches (*Struthio camelus*) kept in pens. *Deutsche Tierärztliche Wochenschrift*, 107:339–341.
- Samraus HH (1994b). Das sexualverhalten des Afrikanischen Staußes (*Struthio camelus*). *Tierärztliche Prax*, 22: 538–541.
- Samraus HH (1995). Die soziale rangordnung in einigen gruppen afrikanischer strauße (*Struthio camelus*) in Namibia. *Journal of Animal Breeding and Genetics*, 112: 234–238.
- Samson J (1996). Behavioural problems of farmed ostriches in Canada. *Canadian Veterinary Journal*, 37:412–414.
- Sauer EG and Sauer EM (1966a). The behaviour and ecology of the South African ostrich. *Living Bird*, 5:45–75.
- Sauer EGF (1970). Interspecific behaviour of the South African ostrich. *Ostrich*, Supply 8: 91-103.
- Sauer EGF (1972). Aberrant sexual behaviour in the South African ostrich. *Auk*, 89:717–737.
- Sauer EGF and Sauer EM (1966b) Social behaviour of the South African ostrich *Struthio camelus australis*. *Ostrich Suppl*, 6:183–191.
- Stewart JS (1994). Ostrich behaviour and behavioural problems. *Association of Avian Veterinarians, Proceedings*, 103–109
- Unattributed (2009). “Big Bird: Unusual Nesting in the Ostrich”. Accessed 18 March 2016 at <https://sextraordinary.wordpress.com/2016/04/04/big-bird-unusual-nesting-in-the-ostrich/>
- Unattributed (2012). “ostrich habitat and distribution Ostrich habitat facts and information” <http://www.bioexpedition.com/ostrich-habitat-and-distribution>.
- Williams JB, Siegfried WR, Milton SJ, Adams NJ, Dean WRJ, PLESSIS MA, Jackson S and Nagy KA (1993). Field metabolism, water requirements, and foraging behaviour of wild ostriches in the Namib. *Ecology*, 74:390–404.





# Performances of Broiler Chickens Fed on Diet Supplemented with Thyme and Oregano Essential Oils Stabilized in a Plant Charcoal Matrix

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## ABSTRACT

This study was designed to mitigate the volatile and oxidative ability of essential oils (EOs) in poultry feed using natural plant charcoal. The dietary treatments consisted of supplementing control diet (R<sub>0</sub>) with 0.01% of the mixture (1/1) of thyme and oregano EOs (R<sub>Th+Or</sub>), 0.2% of *Canarium* charcoal without EO (R<sub>OC</sub>), 0.2% charcoal respectively enriched with 0.01% of thyme EO (R<sub>OC+Th</sub>), oregano EO (R<sub>OC+Or</sub>) and the mixture of EOs (R<sub>OC+Th+Or</sub>). Results revealed a non-significant increase in weight gain for about 5 and 6%, respectively with the mixture of the EOs without charcoal and charcoal enriched with the mixture of the EOs compared to the control (R<sub>0</sub>). The carcass yield was higher with oregano EO and the mixture of EOs compared to the other treatments. Intestinal density was lower (P<0.05) with the mixture of the EOs compared to thyme EO alone and the control ration. Charcoal containing EOs significantly increased (P<0.05) total protein in serum content, triglycerides, albumin, globulin and decreased serum content in creatinin, ASAT, ALAT and cholesterol. Hematological parameters were not significantly affected by the treatments. The blend of EOs associated or not to charcoal increased lactic acid bacteria count in both the ileum and the caecum as compared to *E. coli* and salmonella. It was concluded that *Canarium* charcoal can be used to stabilize EOs in the feed for gut microbiota modulation and better growth performances of broiler chickens.

**Key words:** Broiler, Essential oil, Hematology, Gut microbiota, Oregano, Plant charcoal, Thyme

## INTRODUCTION

Antibiotic Growth Promoters (AGPs) have been banned in several countries of the world because of the resistances developed by bacteria and the presence of the chemical residues in the livestock products which could have harmful consequences for the consumers (Vicente et al., 2007). Many alternatives to the AGPs have been identified and are already sold as food additives for livestock. Among these alternatives we can list the probiotics, prébiotics, organic acids, plants extracts and Essential Oils (EOs).

Essential oils are odorous compounds, generally of complex composition that give plants their color and scent.

The studies of Bolukbasi et al. (2006), Cross et al. (2007) and Khattak et al. (2014) revealed that EOs stimulated feed intake, improve weight gain and offer health advantages to poultry. One of the main mechanisms which explain the improvement of poultry performances is the ability of EOs to balance the gut microbiota by inhibiting pathogens bacterial growth due to their antibacterial properties. The antibacterial activity of EOs is due to the presence of several active compounds such as the carvacrol, thymol, eugenol or cinnamaldéhyde (Mathlouthi et al., 2009; Al-Shuwaili et al., 2015; Moukette et al., 2015). These compounds interact with and increase the permeability of the bacteria cell membrane, deteriorate the enzymatic systems and inhibit or destroy the genetic

material of the bacteria (Hulin et al., 1998; Krishan and Narang, 2014). Another responsible mechanism could be the stimulation of feed, and the secretion of the digestive enzymes of the host (Bento et al., 2013), and the modulation of the immune system which offer healthy performance benefits to poultry (Brenes and Roura, 2010; Tihihonen et al., 2010; Amerah et al., 2011; Hosseini et al., 2013; Khattak et al., 2014; Karadas et al., 2014). *In vitro* studies have concluded that the combination of individual EOs has a greater antibacterial effect than individual EOs alone, indicating a synergy between essential oils of different composition and origin (Bento et al., 2013).

Despite all the beneficial properties mentioned above, using EOs in animal feed are still very problematic due to their instability. Their active compounds are volatile and large quantities of EOs are easily lost during feed processing and storage. Incorporation in a stable matrix can overcome the technical issues of stability in feed. Moreover, incorporation in a stable matrix can help to avoid degradative reactions and the loss of EOs quality leading to flavor optimization of feed, better handling, increase stability, delay release and then enhance the bioavailability of EOs in the digestive tract. The activated charcoal has the ability to bind a variety of substances. This property can be exploited to stabilize essential oils in animal feed, to facilitate their transport and their release in the target sites along the digestive tract where they can optimize the development of beneficial bacteria to chicken. According to Kana et al. (2011), the inclusion of 0.2% of *Canarium schweinfurthii* seeds charcoal in feed increased the weight of broiler chickens for about 12%. The growth promoting effect of this charcoal can be more significant when associated with essential oils.

The present is proposed to give an overview on the potential of plant charcoal to retain and protect essential oil bioactivity, and to evaluate the benefits of the association charcoal-essential oils towards the support of a positive gut bacteria growth and production performances of broiler chickens.

## MATERIALS AND METHODS

### Site of Study

The study was conducted at the poultry unit of the Teaching and Research Farm of the University of Dschang, Cameroon. This farm is located at 5°26' North and 10°26' EST and at an altitude of 1420 m above sea level. Annual temperatures vary between 10°C and 25°C. Rainfall ranges from 1500-2000 mm per annum over a 9 months rainy season (March to November).

### Ethical approval

This study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were humanly handled in respect of the ethical standards laid down in 1964 Declaration of Helsinki and its later amendments.

### Essential oils and charcoal

Essential oils of thyme and oregano were obtained from Barij Esans Company, Tehran, Iran. Mature black fruit seeds (*Canarium schweinfurthii* Engl.) were collected in the villages around the experimental University farm. These seeds were burnt on a wire netting using firewood to obtain black charcoal, it was then quenched with water and sun-dried.

After drying, the charcoal was grounded and sieved to pass through a 1-mm mesh, and lastly used to bind and stabilize essential oils in the experimental rations as follow: Oregano (10g) and Thyme (10g) EOs or their mixture (5g/5g) was respectively mixed with 10g of tween 20 and introduced in 100ml sterile distilled water under constant stirring. After 10 minutes of stirring, 200mg of charcoal was introduced in this solution (EO + tween 20 + distilled water) and homogenized by hand shaking with the hand for 5 minutes to allow the charcoal to absorb all the solution. This EO loaded-charcoal was dried at 55°C for 48 hours and sealed in a nylon bag to prevent air exchanges between the charcoal and the storage environment. This EO loaded-charcoal was used as feed additive with the final EO concentration of 0.01% (10g of EO/100kg of feed).

### Animal

320 days-old Cobb500 broiler chicks were acquired from a local hatchery and divided into 5 experimental groups of 64 chicks each. Each group was subdivided into four replicates of 16 chicks (8 males and 8 females in each replicate). Chicks were litter-brooded to 21 days of age at a density of 20 chicks/m<sup>2</sup>. Vaccination and other routine poultry management practices were maintained. Chicks were weighed at the beginning of the experiment and on a weekly basis thereafter. Feed and water were offered *ad libitum*.

### Dietary treatments and experimental design

At both the starter and finisher phases, a control diet (R0) was formulated (Table 1). The dietary treatments consisted of supplementing control diet (R0) with 0.01% of the mixture (1/1) of thyme and oregano EOs (R<sub>Th+or</sub>), 0.2% of *Canarium charcoal* without EO (R<sub>0C</sub>), 0.2%

charcoal respectively enriched with 0.01% of thyme EO ( $R_{0C+Th}$ ), oregano EO ( $R_{0C+Or}$ ) and the mixture of EOs ( $R_{0C+Th+Or}$ ). Each experimental ration including the control

was fed to 16 chicks (8 males and 8 females) replicated 4 times (4 experimental units) chosen at random in a completely randomized design with 5 treatments.

**Table 1.** Composition of experimental diets

Ingredients (g/kg)	Starter	Finisher
Maize	54	64
Wheat bran	5	1
Soybean meal	22	16
Coton seed meal	5	5
Fish meal	5	5
Borne meal	1	1
Oeister shell	1	1
Palm oil	2	2
Premix 5% *	5	5
<b>Calculated chemical composition</b>		
Metabolizable energy (kcal/kg)	2928.66	3042.76
Crude Protein (g/kg)	23.00	20.40
Lysine (g/kg)	1.43	1.19
Methionine (g/kg)	0.48	0.44
Calcium (g/kg)	1.17	1.35
Phosphorous (g/kg)	0.53	0.56
Crude fibre (g/kg)	5.20	5.14

\*Premix 5%: crude proteins 400mg, Lysin 33mg, Methionin 24 mg, Calcium 80 mg, Phosphorous 20.5 mg, metabolizable energy 2078kcal/kg, Vitamins: Retinol 10 000 000 IU, Cholecalciferol 3 000 000 UI, Tocopherol 2500 IU, Phylloquinon 4000 mg, Thiamin 5000 mg, Riboflavin 500 mg, Pyridoxin 2500 mg, Cyanocobalamin 5 mg, Folic acid 10 000 mg and Niacin 2000 mg.

### Growth, serum biochemical and hematological parameters

Feed intake, weight gain and feed conversion ration were evaluated on a weekly basis in both starter and finisher phases of the study. At the end of the feeding trial at 49 days of age, 10 birds (5 males and 5 females) from each treatment group were randomly selected, fasted for 24 hours and slaughtered for carcass evaluation as preceded by Kana et al. (2017). From each slaughtered chicken, blood was collected in 02 test tubes, one of which contained an anticoagulant. Blood with anticoagulant was used for the hematological analysis using Genius electronic hematocymeter (Model KT-6180, S/N 701106101557, Hong Kong, China). Hematological parameters included White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (HB), Haematocrit (HCT) and Platelets (PLT). Meanwhile, after centrifugation of blood free from anticoagulant, serum was collected and preserved at -20°C for the evaluation of biochemical parameters (total protein, albumin, globulin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total cholesterol, cholesterol HDL and LDL, triglyceride, urea and creatinin) using colorimetric method as prescribed by the commercial kits (Chronolab® kits).

### Microbial count

After slaughtering, the ileum and the cæcum from four birds were sampled per treatment and pooled by intestinal segment. The numbers of lactic acid bacteria, *Escherichia coli* and *Salmonella* were counted on appropriate specific culture medium (MRS Agar for lactic acid bacteria, Mac Conkey AGAR for *E. coli* and SS AGAR for *Salmonella* respectively) as proceeded by Pineda et al. (2012).

### Statistical analysis

All the data were submitted for analysis of the variance using Statistical Package for Social Science (SPSS 21.0) software. Significant differences between treatment means were indicated using Duncan's multiple range tests at 5 % threshold significance (Vilain, 1999).

## RESULTS

Feed Intake (FI), Live Body Weight (LBW), Weight Gain (WG) and gain/feed ratio as affected by thyme and oregano EO and their mixture stabilized in *Canarium* charcoal are summarized in table 2. Feed intake was not markedly affected by the treatments during the starter

phase. Supplementing the diet with the mixture of EOs and the mixture of EOs mixed with charcoal resulted in a non significant increase of WG for about 5 and 6%, respectively. The mixture of EOs also induced a significant decrease ( $P < 0.05$ ) in gain/feed ratio compared to the diet supplemented with charcoal without any EOs and charcoal enriched with oregano EO.

The effects of the various treatments on the carcass yield and the relative weight of organs and cut-out are summarized in table 3. Apart for the relative weight of abdominal fat which significantly decreased with charcoal enriched with the EOs or not, treatments failed to induce any marked effect on carcass yield and the relative weight of legs, head, heart and liver.

Table 4 summarized the development of digestive organs of chickens as affected by charcoal enriched with thyme and oregano EOs. Supplementing the diet with charcoal enriched with thyme EO and the mixture of the EOs lead to a significant decrease ( $P < 0.05$ ) in the relative weight of the pancreas. Charcoal enriched with the mixture of the EOs also induced a significant decrease ( $P < 0.05$ ) in intestinal density. Intestine length and density (weight/length) were not markedly affected by charcoal and EOs.

The effect of experimental diets on the microbial load in the ileum and cæcum are summarized in table 5. Irrespective to the bacterial species, bacterial count markedly increased with charcoal without EO and

charcoal enriched with the EOs in both the ileum and the cæcum compared to the control diet. Regarding the bacterial species, the feeding of broilers with charcoal enriched with thyme and oregano EOs resulted in a significant increase in the number of lactic acid bacteria colonies as compared to *salmonella* and *E. coli* in the cæcum. In the ileum, the increase in lactic acid bacteria count also reached statistical significance with the mixture of EOs.

Table 6 summarized the effect of enrichment of charcoal with EOs on the serum biochemical parameters. Apart for the urea content which was not markedly affected, experimental diets significantly affected all the studied biochemical parameters. Feeding broilers with charcoal without EO and charcoal enriched with Eos resulted in a marked decrease in ALAT and creatinine. The reverse tendency was recorded in the total cholesterol, triglyceride, total protein, albumin and globulin content which significantly ( $P < 0.05$ ) increased with charcoal alone and charcoal enriched with EOs.

Table 7 indicates the effect of experimental diets on hematological parameters of broiler chickens at 49 days of age. As shown in the mentioned table, the hematological values recorded in the present study indicates no significant ( $P > 0.05$ ) impact of charcoal and EOs on red and white blood cell counts, hemoglobin content and hematocrit percentage.

**Table 2.** Growth performances of broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal from one to 49 days old

Study periods (days)	Treatments						SEM	P
	R <sub>0</sub>	R <sub>Th+Or</sub>	R <sub>0C</sub>	R <sub>0C+Th</sub>	R <sub>0C+Or</sub>	R <sub>0C+Th+Or</sub>		
Feed intake (g)								
01 - 21	1416.40 <sup>a</sup>	1347.83 <sup>a</sup>	1428.39 <sup>a</sup>	1379.09 <sup>a</sup>	1426.80 <sup>a</sup>	1422.93 <sup>a</sup>	12.95	0.39
22 - 49	4038.70 <sup>b</sup>	4460.02 <sup>a</sup>	4473.86 <sup>a</sup>	4505.32 <sup>a</sup>	4592.40 <sup>a</sup>	4353.52 <sup>a</sup>	48.02	0.00
01 - 49	5455.10 <sup>b</sup>	5807.85 <sup>a</sup>	5873.38 <sup>a</sup>	5869.34 <sup>a</sup>	5952.30 <sup>a</sup>	5711.52 <sup>ab</sup>	118.59	0.01
Live body weight (g)								
01 - 21	751.72 <sup>b</sup>	808.12 <sup>a</sup>	781.60 <sup>a</sup>	785.94 <sup>a</sup>	796.46 <sup>a</sup>	795.48 <sup>a</sup>	6.02	0.10
01 - 49	2657.4 <sup>a</sup>	2799.27 <sup>a</sup>	2714.67 <sup>a</sup>	2741.22 <sup>a</sup>	2762.97 <sup>a</sup>	2813.10 <sup>a</sup>	21.17	0.31
Body weight gain (g)								
01 - 21	709.72 <sup>b</sup>	766.12 <sup>a</sup>	739.60 <sup>a</sup>	743.94 <sup>a</sup>	754.46 <sup>a</sup>	753.48 <sup>a</sup>	6.02	0.10
22 - 49	1905.71 <sup>a</sup>	1991.14 <sup>a</sup>	1933.07 <sup>a</sup>	1955.28 <sup>a</sup>	1966.51 <sup>a</sup>	2016.83 <sup>a</sup>	18.34	0.60
01 - 49	2615.43 <sup>a</sup>	2757.27 <sup>a</sup>	2672.67 <sup>a</sup>	2699.22 <sup>a</sup>	2720.9 <sup>a</sup>	2770.31 <sup>a</sup>	21.17	0.31
Gain/feed ratio								
01 - 21	2.00 <sup>a</sup>	1.76 <sup>b</sup>	1.90 <sup>ab</sup>	1.83 <sup>b</sup>	1.80 <sup>b</sup>	1.80 <sup>b</sup>	0.024	0.04
22 - 49	2.12 <sup>c</sup>	2.24 <sup>ab</sup>	2.32 <sup>ab</sup>	2.31 <sup>ab</sup>	2.34 <sup>a</sup>	2.16 <sup>c</sup>	0.025	0.05
01 - 49	2.09 <sup>ab</sup>	2.10 <sup>ab</sup>	2.20 <sup>a</sup>	2.18 <sup>ab</sup>	2.19 <sup>a</sup>	2.06 <sup>b</sup>	0.017	0.08

a,b,c: Means with the same superscript on the same line are not significantly different ( $P > 0.05$ ). P= probability. R<sub>0</sub>=control ration without any supplement; R<sub>Th+Or</sub>=R<sub>0</sub>+0.01% mixture of EOs; R<sub>0C</sub> = R<sub>0</sub> + 0.2% charcoal; R<sub>0C+Th</sub> = R<sub>0</sub> + 0.2% charcoal + 0.01% Thyme EO; R<sub>0C+Or</sub> = R<sub>0</sub> + 0.2% + 0.01% Oregano EO; R<sub>0C+Th+Or</sub> = R<sub>0</sub> + 0.2% of charcoal + 0.01% mixture of EOs.

**Table 3.** Carcasses yield (%) and the relative weight of organs and cuts out (%) of broiler chicken as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal at 49 days old

Carcass traits (%)	Treatments						SEM	P
	R <sub>0</sub>	R <sub>Th+Or</sub>	R <sub>0C</sub>	R <sub>0C+Th</sub>	R <sub>0C+Or</sub>	R <sub>0C+Th+Or</sub>		
Carcasse yield (%)	73.09 <sup>b</sup>	74.48 <sup>ab</sup>	73.57 <sup>ab</sup>	73.8 <sup>ab</sup>	74.4 <sup>ab</sup>	74.73 <sup>a</sup>	0.21	0.16
Legs (%BW)	3.45 <sup>a</sup>	3.80 <sup>a</sup>	3.64 <sup>a</sup>	3.56 <sup>a</sup>	3.54 <sup>a</sup>	3.80 <sup>a</sup>	0.06	0.62
Head (%BW)	1.92 <sup>a</sup>	1.84 <sup>a</sup>	1.98 <sup>a</sup>	1.86 <sup>a</sup>	1.95 <sup>a</sup>	1.93 <sup>a</sup>	0.02	0.78
Heart (%BW)	0.51 <sup>a</sup>	0.53 <sup>a</sup>	0.53 <sup>a</sup>	0.47 <sup>ab</sup>	0.39 <sup>b</sup>	0.50 <sup>a</sup>	0.02	0.07
Liver (%BW)	1.67 <sup>a</sup>	1.44 <sup>a</sup>	1.49 <sup>a</sup>	1.55 <sup>a</sup>	1.62 <sup>a</sup>	1.63 <sup>a</sup>	0.03	0.23
Abdominal fat (%BW)	2.40 <sup>a</sup>	1.86 <sup>b</sup>	1.67 <sup>b</sup>	1.55 <sup>b</sup>	2.02 <sup>ab</sup>	1.62 <sup>b</sup>	0.09	0.00

<sup>a,b,c</sup> Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. R<sub>0</sub>=control ration without any supplement; R<sub>Th+Or</sub>=R<sub>0</sub>+0.01% mixture of EOs; R<sub>0C</sub> = R<sub>0</sub> + 0.2% charcoal; R<sub>0C+Th</sub> = R<sub>0</sub> + 0.2% charcoal + 0.01% Thyme EO; R<sub>0C+Or</sub>= R<sub>0</sub> + 0.2% + 0.01% Oregano EO; R<sub>0C+Th+Or</sub>= R<sub>0</sub> + 0.2% of charcoal + 0.01% mixture of EOs.

**Table 4.** Relative weight of digestion organs of broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal at 49 days old

Digestive organs traits	Treatments						SEM	P
	R <sub>0</sub>	R <sub>Th+Or</sub>	R <sub>0C</sub>	R <sub>0C+Th</sub>	R <sub>0C+Or</sub>	R <sub>0C+Th+Or</sub>		
Gizzard (% BW)	1.59 <sup>a</sup>	1.57 <sup>a</sup>	1.48 <sup>a</sup>	1.60 <sup>a</sup>	1.62 <sup>a</sup>	1.47 <sup>a</sup>	0.03	0.42
Pancréas (% BW)	0.26 <sup>a</sup>	0.21 <sup>ab</sup>	0.20 <sup>abc</sup>	0.13 <sup>c</sup>	0.19 <sup>abc</sup>	0.16 <sup>bc</sup>	0.01	0.02
Intestin weight (g)	84.08 <sup>a</sup>	78.10 <sup>bc</sup>	80.30 <sup>abc</sup>	87.20 <sup>ab</sup>	77.90 <sup>a</sup>	74.20 <sup>c</sup>	1.75	0.02
Intestin length (cm)	210.08 <sup>a</sup>	204.60 <sup>a</sup>	204.50 <sup>a</sup>	204.70 <sup>a</sup>	202.10 <sup>a</sup>	205.20 <sup>a</sup>	2.71	0.96
Intestin density (g/cm)	0.43 <sup>a</sup>	0.38 <sup>ab</sup>	0.39 <sup>ab</sup>	0.43 <sup>a</sup>	0.39 <sup>ab</sup>	0.36 <sup>b</sup>	0.01	0.03

<sup>a,b,c</sup> Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. R<sub>0</sub>=control ration without any supplement; R<sub>Th+Or</sub>=R<sub>0</sub>+0.01% mixture of EOs; R<sub>0C</sub> = R<sub>0</sub> + 0.2% charcoal; R<sub>0C+Th</sub> = R<sub>0</sub> + 0.2% charcoal + 0.01% Thyme EO; R<sub>0C+Or</sub>= R<sub>0</sub> + 0.2% + 0.01% Oregano EO; R<sub>0C+Th+Or</sub>= R<sub>0</sub> + 0.2% of charcoal + 0.01% mixture of EOs.

**Tableau 5.** Ileal and cæcal microbial load of 49 days old broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal

Bacterial count Log <sub>10</sub> (10 <sup>7</sup> cfu/ml)	Treatments						SEM	P
	R <sub>0</sub>	R <sub>Th+Or</sub>	R <sub>0C</sub>	R <sub>0C+Th</sub>	R <sub>0C+Or</sub>	R <sub>0C+Th+Or</sub>		
Ileum								
Lactic acid bacterial	8.48 <sup>cA</sup>	9.28 <sup>bA</sup>	9.30 <sup>bC</sup>	9.43 <sup>bA</sup>	9.73 <sup>aA</sup>	9.90 <sup>aA</sup>	0.09	0.00
<i>E. coli</i>	7.96 <sup>cA</sup>	8.64 <sup>bB</sup>	9.82 <sup>aA</sup>	8.88 <sup>bB</sup>	8.80 <sup>bB</sup>	8.55 <sup>bcC</sup>	0.15	0.00
<i>Salmonella</i>	7.72 <sup>cA</sup>	8.72 <sup>bB</sup>	9.58 <sup>aB</sup>	8.67 <sup>bC</sup>	9.71 <sup>aA</sup>	9.05 <sup>bB</sup>	0.15	0.00
SEM	0.19	0.10	0.07	0.11	0.15	0.19	--	--
p	0.300	0.011	0.000	0.000	0.000	0.000	--	--
Cæcum								
Lactic acid bacterial	8.13 <sup>cA</sup>	9.43 <sup>abA</sup>	9.52 <sup>abA</sup>	9.36 <sup>bA</sup>	9.20 <sup>bA</sup>	9.93 <sup>aA</sup>	0.13	0.00
<i>E. coli</i>	7.00 <sup>dB</sup>	7.81 <sup>abC</sup>	8.75 <sup>aB</sup>	7.54 <sup>cC</sup>	8.21 <sup>bB</sup>	8.54 <sup>aB</sup>	0.12	0.00
<i>Salmonella</i>	7.00 <sup>dB</sup>	8.37 <sup>cB</sup>	9.55 <sup>aA</sup>	8.76 <sup>bB</sup>	8.35 <sup>cB</sup>	8.19 <sup>cC</sup>	0.16	0.00
SEM	0.19	0.11	0.07	0.11	0.14	0.20	--	--
P	0.000	0.000	0.001	0.000	0.005	0.000	--	--

<sup>a,b,c,d</sup> Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. A,B: Means with the same superscript on the same column are not significantly different (P>0.05). R<sub>0</sub>=control ration without any supplement; R<sub>Th+Or</sub>=R<sub>0</sub>+0.01% mixture of EOs; R<sub>0C</sub> = R<sub>0</sub> + 0.2% charcoal; R<sub>0C+Th</sub> = R<sub>0</sub> + 0.2% charcoal + 0.01% Thyme EO; R<sub>0C+Or</sub>= R<sub>0</sub> + 0.2% + 0.01% Oregano EO; R<sub>0C+Th+Or</sub>= R<sub>0</sub> + 0.2% of charcoal + 0.01% mixture of EOs.

**Table 6.** Serum biochemical parameters of 49 days old broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal

Biochemical parameters	R <sub>0</sub>	R <sub>Th+Or</sub>	R <sub>0+C</sub>	R <sub>0+Th</sub>	R <sub>C0+Or</sub>	R <sub>C0+Th+Or</sub>	SEM	P
ASAT (U/L)	42,00 <sup>ab</sup>	26.25 <sup>b</sup>	38,06 <sup>ab</sup>	31,50 <sup>b</sup>	52,50 <sup>a</sup>	34,13 <sup>b</sup>	2.59	0.19
ALAT (U/L)	32,38 <sup>a</sup>	15.31 <sup>bc</sup>	30,28 <sup>a</sup>	8,17 <sup>c</sup>	10,21 <sup>bc</sup>	18,20 <sup>b</sup>	2.16	0.00
Creatinine (mg/dl)	2,63 <sup>a</sup>	2.02 <sup>b</sup>	1,67 <sup>bc</sup>	2,79 <sup>a</sup>	1,46 <sup>c</sup>	0,61 <sup>d</sup>	0.16	0.00
Urea (mg/dl)	7,09 <sup>b</sup>	7.06 <sup>b</sup>	7,34 <sup>ab</sup>	7,78 <sup>a</sup>	7,09 <sup>b</sup>	7,63 <sup>ab</sup>	0.09	0.06
Total cholesterol (mg/dl)	62,77 <sup>bc</sup>	81.82 <sup>a</sup>	89,08 <sup>a</sup>	76,20 <sup>ab</sup>	78,36 <sup>a</sup>	60,31 <sup>c</sup>	2.59	0.00
HDL-cholesterol (mg/dl)	30,94 <sup>ab</sup>	29.37 <sup>ab</sup>	23,18 <sup>b</sup>	27,51 <sup>b</sup>	43,71 <sup>a</sup>	19,41 <sup>b</sup>	2.25	0.02
LDL-cholesterol (mg/dl)	27,63 <sup>ab</sup>	19.29 <sup>b</sup>	11,52 <sup>b</sup>	17,40 <sup>b</sup>	38,69 <sup>a</sup>	11,02 <sup>b</sup>	1.70	0.00
Triglyceride (mg/dl)	13,70 <sup>c</sup>	19.09 <sup>bc</sup>	24,07 <sup>b</sup>	20,90 <sup>b</sup>	22,33 <sup>b</sup>	36,29 <sup>a</sup>	2.08	0.00
Total protein (g/dl)	2,26 <sup>b</sup>	2.34 <sup>b</sup>	2,47 <sup>b</sup>	2,40 <sup>b</sup>	2,94 <sup>a</sup>	2,96 <sup>a</sup>	0.07	0.00
Albumin (g/dl)	1.20 <sup>c</sup>	2.10 <sup>cb</sup>	2.83 <sup>b</sup>	3.8 <sup>a</sup>	3.93 <sup>a</sup>	2.14 <sup>cb</sup>	0.24	0.00
Globulin (g/dl)	0.80 <sup>b</sup>	1.48 <sup>ab</sup>	0.77 <sup>b</sup>	1.05 <sup>b</sup>	1.07 <sup>b</sup>	1.72 <sup>a</sup>	0.35	0.13

<sup>a,b,c</sup>: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. R<sub>0</sub>=control ration without any supplement; R<sub>Th+Or</sub>=R<sub>0</sub>+0.01% mixture of EOs; R<sub>0C</sub> = R<sub>0</sub> + 0.2% charcoal; R<sub>0C+Th</sub> = R<sub>0</sub> + 0.2% charcoal + 0.01% Thyme EO; R<sub>0C+Or</sub> = R<sub>0</sub> + 0.2% + 0.01% Oregano EO; R<sub>0C+Th+Or</sub> = R<sub>0</sub> + 0.2% of charcoal + 0.01% mixture of EOs. ASAT: Aspartate aminotransferase, ALAT: Alanine aminotransferase

**Table 7.** Blood parameters of 49 days old broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal

Blood parameters	Treatments						SEM	P
	R <sub>0</sub>	R <sub>Th+Or</sub>	R <sub>0C</sub>	R <sub>0C+Th</sub>	R <sub>0C+Or</sub>	R <sub>0C+Th+Or</sub>		
WBC (10 <sup>3</sup> /μl)	87.52	90.03	83.27	84.68	84.15	88.98	0.80	0.05
RBC (10 <sup>6</sup> /μl)	2.82	2.97	2.80	2.84	2.80	3.09	0.04	0.58
Hb (g/dl)	14.03	14.66	13.82	13.73	14.43	14.85	0.18	0.44
HCT (%)	33.98	36.28	33.15	33.00	34.48	36.55	0.73	0.67
MCV (fL)	120.18	122.21	118.48	116.27	122.98	118.40	1.04	0.42
MCH (pg)	49.80	49.36	49.48	48.35	52.68	48.07	0.63	0.36
PLT (10 <sup>3</sup> /μl)	27.83	46.83	38.17	29.83	27.83	38.25	2.71	0.23

WBC = White blood cell; RBC = red blood cell; Hb = Hemoglobin; HCT = Hematocrit; MCV = mean globular volume; MCH = Mean concentration of hemoglobin; PLT = Blood plate. R<sub>0</sub>=control ration without any supplement; R<sub>Th+Or</sub>=R<sub>0</sub>+0.01% mixture of EOs; R<sub>0C</sub> = R<sub>0</sub> + 0.2% charcoal; R<sub>0C+Th</sub> = R<sub>0</sub> + 0.2% charcoal + 0.01% Thyme EO; R<sub>0C+Or</sub> = R<sub>0</sub> + 0.2% + 0.01% Oregano EO; R<sub>0C+Th+Or</sub> = R<sub>0</sub> + 0.2% of charcoal + 0.01% mixture of EOs.

## DISCUSSION

In the present study, charcoal and EOs significantly (P < 0.05) enhanced feed intake in the finisher phase and throughout the experimental period as compared to the control diet without any supplement. Similar to this result, Kana et al. (2011) and Khattak et al. (2014) respectively reported that *Canarium* charcoal and blend of EOs stimulated feed intake, improved weight gain and offered health advantages to poultry. The mixture of EOs with charcoal resulted in a non significant increase in weight gain for about 6% compared to the control diet without charcoal and EOs. This result is in close agreement with Lee et al. (2003) and Jang et al. (2007) who reported no significant effect on the weight gain and live body weight

of broilers fed on a commercial feed additive containing thymol and cinnamaldehyde. The upward trend noticed in weight gain in the present study could be due to the antibacterial activity of carvacrol and thymol, the main active compounds of oregano and thyme EOs which might modulate the gut microbiota by inhibiting pathogenic bacterial growth due to their selective antibacterial properties. A more balanced microbiota population in gut could lead to a better efficiency in digestibility of nutrients, resulting in growth enhancement (Toghyani et al., 2010; Khattak et al., 2014). The weight gain improvement of broilers achieved in the present study could also be attributed to the positive effect of charcoal and EOs on nutrient digestibility through the stimulation of the digestive enzymes of the host as reported by Kana et



al. (2011), Bento et al. (2013) and Parakesvakis et al. (2015) or by their antioxidant effects in the intestine (Karadas et al., 2014).

The mixture of EOs with charcoal induced a significant decrease ( $P < 0.05$ ) in gain/feed ratio as compared to charcoal an individual's EO alone, suggesting a synergy between active compounds of oregano and thyme EOs. A number of *in vitro* studies also provided evidence of a synergy between EOs with different composition and origin (Malthlouthi et al., 2010; Bento et al., 2013). The enhancement of gain/feed ratio recorded in the present study suggested that the digestibility of feed and the absorption of the nutriment are better in chickens fed on diets supplemented with charcoal and charcoal enriched with EOs.

The feeding of broilers with charcoal enriched with thyme and oregano EOs resulted in a significant decrease in abdominal fat deposit. This low deposit of abdominal fat could be due to the effect of carvacrol and thymol present in these EOs on the metabolism of fat. Indeed, according to Zhang et al. (2007) the activation of the transient receptor (TRPV1) by active compounds like capsaicin found in some plants prevented the deposition of fat in mice and humans. It might be the case with carvacrol and thymol and other major compounds like linloul, p-cymene,  $\alpha$  and  $\gamma$ -terpinene (Malthlouthi et al., 2010) found in thyme and oregano EOs used in the present study. The low fat deposit recorded here can explain the decrease in relative weight of the pancreas, suggesting the reduction in hormonal secretions by this organ with the enrichment of the charcoal by the EOs.

The intestine density (weight/length) which is an indication of villi size of the mucosa layer markedly decreased with the charcoal and EOs as compared to the control diet. This result agrees with the findings of Abdel-Fatter et al. (2008) and Kana et al. (2011) who respectively reported that the inclusion of organic acids and the charcoal from *Canarium schweinfurthii* seeds and maize cob in broilers diet tend to decrease the intestine density.

Irrespective to the bacteria species, bacteria count increased markedly with charcoal without EO and charcoal enriched with the EOs both in the ileum and cæcum compared to the control diet. Whatever the case, the numbers of lactic acid bacteria (beneficial bacteria) count were higher than *salmonella* and the *E. coli* (pathogens). This situation can explain the improvement in weight gain recorded in this study. The present result is in agreement with the findings of Lan et al. (2005) and Murry et al. (2006) who reported an increase in lactic acid bacteria population in the gut of healthy chickens. When

the living conditions in the intestine is favorable, the lactic acid bacteria multiply and eliminate pathogenic bacteria (*Salmonella* and *Escherichia coli*) by acidifying the milieu and producing antibacterial substances like organic acids (Elaroussi et al., 2008). Although the proliferation of lactic acid bacteria stimulates the immune system of the chickens (Tiihonen et al., 2010; Amerah et al., 2011; Khattak et al., 2014), it was shown that EOs prevent the adhesion of the pathogens bacteria on their intestinal mucosa by stimulating the secretion of mucus (Jamroz et al., 2006).

This study revealed that feeding broilers with charcoal without EO and charcoal enriched with EOs resulted in an increase in serum content of cholesterol, triglyceride, total protein, albumin and globulin. The result of this study confirmed the findings of Kana et al. (2011) who reported the beneficial effects of dietary *Canarium* and maize cob charcoals on hematological and biochemical parameters in broilers. The increase in serum content of protein suggested the capacity of EOs to improve digestion and absorption of proteins as previously reported by Bento et al. (2013) and Krishan and Narang (2014) allowing a better use of protein in broiler chicken and thus an improvement of the weight gain. The decrease in HDL-cholesterol and LDL-cholesterol recorded in this study agrees with the results of Ali et al. (2007) who reported that the addition of thyme in the diet of chicken induce a significant decrease in the serum content in HDL-cholesterol and total cholesterol. The decrease in cholesterol content recorded in this study could be due to the inhibiting effects of thymol and carvacrol on HMG-CoA reductase, a key enzyme in cholesterol synthesis (Crowell, 1999). The enrichment of charcoal with thyme and oregano EOs and their mixture markedly decreased the serum content in ALAT. The present result contradicted the findings of Khattak et al. (2014) who recorded no significant effect on the serum content in ASAT and ALAT of broilers with the commercial product containing thymol, carvacrol, cinnamaldehyde, oregano, peppermint and pepper.

Blood parameters reflect the healthy state of an organism and any changes happening to it could be an indication of unbalance feeding or disease attack. This study revealed that feeding broilers with charcoal enriched with thyme and oregano EOs did not have any significant effect on blood parameters. This finding is in close agreement with Kana et al. (2011) and Toghyani et al. (2010) who respectively reported that supplementing broiler chickens with *Canarium* and maize cob charcoals, and thyme powder did not have any marked effect on

white and red blood cells counts, hemoglobin content and hematocrit percentage.

## CONCLUSION

The result presented in the present study suggested that charcoal from *Canarium schweinfurthii* seeds can be used to entrap, stabilize and facilitate the incorporation of essential oils in poultry feed for gut microbiota modulation and for a better growth rate without any detrimental effect on serum and hematological parameters.

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## Competing interests

The authors declare that they have no competing interests.

## Author's contributions

Ngouana, Ncedem, Kuiede and Yemdjie went to the field to carry out the research and collect the samples. Kana supervised the overall research work. Ngouana wrote the first draft before being revised by Kana, Meimandipour and Teguaia, and approved by all the authors.

## Consent to publish

All persons gave their informed consent prior to their inclusion in the study.

## REFERENCES

- Abdel Fattah SA, El-Sanhoury MH, El-Mednay NM and Abdel-Azem F (2008). Tyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. *International Journal of Poultry Science*, 7 (3): 215-222.
- Al-Shuwaili MA, Ibrahim IE and Naqi Al-Bayati MT (2015). Effect of dietary herbal plants supplement in turkey diet on performance and some blood biochemical parameters. *Global Journal of Bio-science and Biotechnology*, 4 (2), 153-157
- Amerah AM, Péron A, Zaefarian F and Ravindran V (2011). Influence of whole wheat inclusion and a blend of essential oils on the performance, nutrient utilisation, digestive tract development and ileal microbiota profile of broiler chickens. *British Poultry Science*, 52: 124-132. DOI: 10.1080/00071668.2010.548791
- Bento MHL, Ouwehand AC, Tiihonen K, Lahtinen S, Nurminen P, Saarinen MT, Schulze H, Mygind T and Fisher J (2013). Essential oil and their use in animal feeds for monogastric animals – effect on feed quality, gut microbiota, growth performance and food safety: a review. *Veterinarni Medicina*, 58 (9): 449-458. Available at: <http://vri.cz/docs/vetmed/58-9-449.pdf>
- Bölükbaşı SC, Erhan MK and Özkan A (2006). Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers. *South Africa Journal Animal Science*, 36: 189-196.
- Brenes A and Roura E (2010). Essential oils in poultry nutrition: Main effects and modes of action. *Animal Feed Science and Technology*, 158: 1-14. DOI: 10.1016/j.anifeedsci.2010.03.007
- Cross DE, Mcdevitt RM, Hillman K and Acamovic T (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. *British Poultry Science*, 48(4):496-506.
- Crowell PL (1999). Prevention and therapy of cancer by dietary monoterpenes. *The Journal of Nutrition*, 129: 775- 778.
- Elaroussi MA, Mohamed MS, El-Barkouki EM, Abdou AM and Hatab MH (2008). Ochratoxicosis in Broiler Chickens: Functional and Histological Changes in Target Organs. *International Journal of Poultry Science*, 7 (5): 414- 422
- Hosseini FS, Zandi M, Rezaei M and Farahmandghavi F (2013). Two step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and *in vitro* release study. *Carbohydrate Polymers*, 95:50-56. DOI: 10.1016/j.carbpol.2013.02.031
- Hulin V, Mathot AG, Mafart P and Dufosse L (1998). Les propriétés antimicrobiennes des huiles essentielles et composés d'arômes. *Sciences des Aliments*, 18: 563-582.
- Jamroz D, Wiertelcki T, Houszka M and Kamel C (2005). 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. *Journal of Animal Physiology and Animal Nutrition*, 90: 255-268.
- Jang IS, Ko YH, Kang SY and Lee CY (2007). Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Animal Feed Science and Technology*, 134: 304-315.
- Jang IS, Yang HY, Ko YH, Ha JS, Kim JY, Kang SY,

- Yoo DH, Nam DS, Kim DH and Lee CY (2004). Influence of Essential Oil Components on Growth Performance and the Functional Activity of the Pancreas and Small Intestine in Broiler Chickens. *Asian-Australian Journal of Animal Science*, 17(3): 394-400
- Kana JR, Teguia A, Mungfu BM and Tchoumboue J (2011). Growth performance and carcass characteristics of broiler chickens fed diets supplemented with graded levels of charcoal from maize cob or seed of *Canarium schweinfurthii* Engl. *Tropical Animal Health Production*, 43: 51-56. DOI: 10.1007/s11250-010-9653-8
- Kana JR, Mube Kuetché H, Ngouana Tadjong R, Tsafong F, Komguez R, Yangoue A and Teguia A (2017). Effect of dietary mimosa small bell (*Dichostachys glomerata*) fruit supplement as alternative to antibiotic growth promoter for broiler chicken. *Journal of World's Poultry Research*, 7(1): 27-34.
- Karadas F, Pirgozliev V, Rose SP, Dimitrov D, Oduguwa O and Bravo D (2014). Dietary essential oils improve the hepatic antioxydative status of broiler chickens. *British Poultry Science*, 1: 22-27.
- Khattak F, Ronchi A, Castelli P and Sparks N (2014). Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. *Poultry Science*, 93: 132-7.
- Krishan G and Narang A (2014). Use of essential oils in poultry nutrition: A new approach. *Journal of Advance Veterinary and Animal Research*, 1: 156-162.
- Lee KW, Everts H, Kappert HJ, Frehner M, Losa R and Beynen AC (2003). Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British Poultry Science*, 44: 450-457.
- Mathlouthi N, Bouzaïenne T, Oueslati I, Recoquillay F, Hamdi M and Bergaoui R (2009). Effet de deux préparations d'huiles essentielles sur la croissance des bactéries *in vitro* et les performances du poulet de chair. INRA (Eds). 8<sup>èmes</sup> Journée de Recherche Avicole, INRA St Malo, 454-458.
- Moukette M, Constant AP, Prosper CNB, Vicky JAM, Eustace B and Jeanne YN (2015). *Afrotyrax lepidophyllus* extracts exhibit in vitro free radical scavenging, antioxidant potential and protective properties against liver enzymes ion mediated oxidative damage. *BMC Research Notes*, 8(344), 1-12. Doi: 10.1186/s13104-015-1304-8
- Murry AC, Hinton A and Buhr (2006). Effect of Botanical Probiotic Containing Lactobacilli on Growth Performance and Populations of Bacteria in the Ceca, cloaca, and Carcass Rinse of Broiler Chickens. *International Journal of Poultry Science*, 5(4): 344-350.
- Pineda L, Chwalibog A, Sawosz E, Lauridsen C, Engberg R, Elnif J, Hotowy A, Sawosz F, Gao Y, Ali A and Sepehri H (2012). Effect of silver nanoparticles on growth performance, metabolism and microbial profile of broiler chickens. *Archives of Animal Nutrition*, 66(5): 416-429. DOI: 10.1080/1745039X.2012.710081
- Paraskevakis N, Tsiplakou E, Daferera D, Sotirakoglou K, Polissiou M and Zevvas G (2015). Changes in essential oil content and composition of *Origanum vulgare* spp. *Hirtum* during storage as a whole plant or after grinding and mixing with a concentrate ruminant diet. *Journal of Essential Oil Research*, 27(3): 264-270. DOI: <http://dx.doi.org/10.1080/10412905.2015.1009562>
- Tiihonen K, Kettunen H, Bento MHL, Saarinen M, Lahtinen S, Ouwehand AC, Schulze H and Rautonen N (2010). The effect of feeding essential oils on broiler performance and gut microbiota. *British Poultry Science*, 51:381-392. DOI: 10.1080/00071668.2010.496446
- Toghyani M, Tohidi M, Gheisari AA and Tabeidian SA (2010). Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. *African Journal of Biotechnology*, 9: 6819-6825. DOI: 10.5897/AJB09.1998
- Vicente JL, Avina L, Torres-Rodriguez A, Hargis B and Tellez G (2007). Effect of a *Lactobacillus* spp-based probiotic culture product on broiler chick performance under commercial conditions. *International Journal of Poultry Science*, 6 (3): 154-156.
- Vilain M (1999). Méthodes expérimentales en Agronomie. Pratique et analyse. Editions Tec et Doc. Paris. 337p
- Zhang GF, Yang ZB, Wang Y, Yang WR, Jiang SZ and Gai GS (2009). Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poultry Science*, 88: 2159-2166. DOI:10.3382/ps.2009-00165



# Effect of Chemically Treated Litter on Ammonia Emission, Performance and Carcass Characteristics of Broiler Chicken

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## ABSTRACT

The condition of litter is a single major factor in deciding the emission of various harmful gases particularly ammonia, which is a major environmental concern, affecting the overall welfare of birds. Therefore, a study was conducted with the objectives to assess the effect of two chemicals namely aluminum sulfate and calcium carbonate on litter ammonia emission, performance and carcass characteristics of broiler chicken. A total of 240 day old Cobb broiler chicks were randomly distributed into four treatment groups, each having 4 replicates of 13 chicks each. In the control group no chemical was added to litter; however, in other groups litter was treated with Aluminum Sulfate (AS) @ 25g/kg; Calcium Carbonate (CC) @ 50g/kg; and combination of 25g Aluminum Sulfate and 50g Calcium Carbonate/kg (ASCC). The results revealed a significant ( $P < 0.05$ ) reduction in litter ammonia emission in AS and ASCC groups compared to control and CC, which in turn had no statistical ( $P > 0.05$ ) difference among themselves. AS was found to be highly effective in reducing the ammonia emission levels, either by itself or in combination, with values of  $9.46 \pm 0.35$  (AS) and  $10.499 \pm 0.39$  (ASCC) compared to  $47.7 \pm 2.40$  and  $51.15 \pm 1.85$  ppm in CC and control. A significant ( $P < 0.05$ ) increase in the Body Weight Gain (BWG) of chicks in AS and ASCC groups with final BWG of 1069.76 g in control, 1358.21 g in AS, 1086.66 g in CC and 1370 g in ASCC. Likewise, an improved FCR of 1.86 was observed in both AS and ASCC groups followed by 1.98 in CC and 1.99 in control. No significant ( $P > 0.05$ ) differences were found with respect to various carcass characteristics among treatment groups as compared to control. In conclusion, compared to CC, AS was found to be highly effective in reducing the litter ammonia emission and improving the performance of birds.

**Key words:** Aluminium sulphate, Ammonia emission, Broiler chicken, Performance

## INTRODUCTION

Poultry production, particularly broiler chicken production is primarily done under a deep litter system having an absorbent material (known as litter) on floor. Most common litter materials used in various parts of the world include softwood and hardwood shavings, sawdust, chopped straws, seeds and hulls, cardboard peat, sand etc.

(Grimes et al., 2002; Lopes et al., 2013). Litter quality plays a significant role because of its effect on bird health, performance parameters, carcass quality and welfare of broilers (Đukić Stojčić et al., 2016). Litter must be kept dry as reported by Ritz et al. (2006) that very wet litter results in high ammonia production which negatively affects productive performance of broilers. Following defecation by birds, the breakdown of fecal matter in litter

occurs, leading to the production of various gaseous pollutants; whose concentration and emission is influenced by the litter type, management, humidity and temperature (Redding, 2013).

Amongst these, ammonia is one such gaseous product which is more harmful for the environment, bird and human health. It is a colorless gas, highly irritating, produced up on chemical and microbial breakdown of uric acid after excretion from the bird (Gates et al., 2005). Ammonia formation primarily occurs by microbial degradation of uric acid in litter particularly under the influence of *Bacillus pasteurii*, which is one of the primary uricolytic bacteria (Bacharach, 1957; Schefferle, 1965). These bacteria reportedly require alkaline pH (around 8.5) for their optimum growth (Elliott and Collins, 1982). Reduced feed intake and growth rate of chicken have been reported once ammonia concentrations increases in poultry sheds (Kristensen et al., 2000). When emitted to the atmosphere, ammonia can rapidly react with acidic compounds and gets converted to aerosolized ammonium particles, thereby influencing ecological balance, biodiversity and water systems (Galloway and Cowling, 2002).

Therefore, there is an utmost need to contain the production and emission of ammonia from poultry houses by various litter amendments like use of acidifiers, alkaline materials, adsorbers, inhibitors, microbes and enzymes (Shah et al., 2006). In view of this negative impact of ammonia and likely benefit litter amendment, a study was conducted with the objectives to assess the effects of two chemicals namely aluminum sulfate and calcium carbonate on litter ammonia emission, performance and carcass characteristics of broiler chicken.

## MATERIALS AND METHODS

### Ethical approval

The experimental protocol was approved by the Institutional Animal Ethics Committee, J&K, India.

### Bird Husbandry and experimental diets

The experiment was conducted at the Research Farm of the Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Srinagar, India. A total of 208 day old Cobb broiler chicks were procured from a reputed source and reared together in battery cages until 7 days of age. On 8<sup>th</sup> day, the chicks were randomly distributed into four treatment groups, each having 4 replicates of 13 chicks. The litter material used was saw

dust which is cheap and readily available in Indian subcontinent. Dry aluminum sulfate and calcium carbonate were procured from the market and added to fresh litter by top dressing onto its surface. The treatment groups were as follows: Control in which no chemical was added to litter; litter treated with Aluminum Sulfate (AS) @ 25g/kg; Calcium Carbonate (CC) @ 50g/kg; and combination of 25g Aluminum Sulfate and 50g Calcium Carbonate/kg (ASCC).

Chicks of each replicate were housed in individual floor pens on deep litter for a period up to 6 weeks of age. Birds had free access to feed (commercially available) and water throughout and were maintained on a constant 24 hours light schedule. All chicks were vaccinated against New castle disease with F<sub>1</sub> strain vaccine and Infectious bursal disease with B<sub>2</sub>K vaccine on 5<sup>th</sup> and 16<sup>th</sup> day of age respectively in accordance with regional veterinary authority. All the treatment groups were maintained in similar rearing conditions as per the standard protocol.

### Ammonia Emission

At the end of trial, litter condition was evaluated. The litter samples from five locations within each pen (four peripheral-equidistant from each pen corner, and one central) were collected and thoroughly mixed to obtain representative sample of the entire pen. The ammonia released from litter samples was determined as per the method of Hernandez and Cazetta (2001) which is based on gaseous ammonia fixation by micro-diffusion. The litter samples from five locations within each pen (four peripheral-equidistant from each pen corner, and one central) were collected and thoroughly mixed to obtain representative sample of the entire pen. Representative sample of 100 g of fresh litter was weighed from each pen every week and placed in a 500 ml cylindrical flask and leveled. A 50 ml beaker containing 10 ml of 2 % boric acid solution was placed on the top of the litter and the flask was closed and incubated for 20 hours at 30° C. The boric acid solution was then titrated against 0.1 N sulfuric acid with methyl orange and bromocresol green indicator. Ammonia released from litter (mg/100g litter) was calculated by multiplying the amount of sulfuric acid used (A) by its normality and the molecular weight of ammonia (17). This released ammonia in mg was converted into ppm/100 g litter as:

$$\text{Ammonia released (ppm / 100 g litter)} = A \times N \times 17 \times 10$$

A = Volume of sulfuric acid spent (ml)

N = Normality of sulfuric acid

17 = Molar mass of ammonia

10 = Conversion coefficient from mg to ppm

### Performance of birds

The body weight of birds was recorded on an individual basis and Body Weight Gain (BWG) was calculated at weekly intervals and the weighed quantity of feed was placed in the feed bins allotted to each replicate and feed was offered adlib from the respective feed bins. At weekly intervals the feed left in the respective bins known as residual feed was weighed again to determine the replicate wise Feed Consumption (FC) during that week. Feed Conversion Ratio (FCR) (i.e. feed: gain in body weight) of birds was worked out at weekly intervals for the entire experimental period by taking into consideration the weekly feed consumption and body weight gain.

### Carcass characteristics

At the end of trial, two birds per replicate were selected at random and utilized for carcass evaluation study. The birds were weighed before fasting and slaughtered by the Halal method and a bleeding time of 2 minutes was allowed. The shanks were cut off at the hock and carcass was subjected to scalding process at 60°C for 30 seconds. The feathers were removed completely by hand picking leaving the skin intact. Thereafter, the abdominal cavity was opened to expose the visceral organs. Slaughter characteristics, yield of giblets and vital organs were calculated as per the method of Salahuddin et al. (2000).

### Statistical analysis

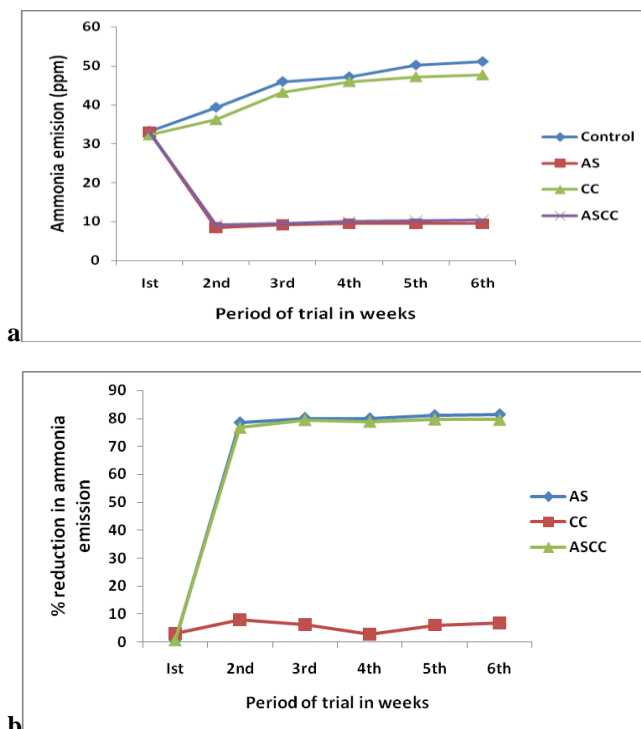
Data generated was grouped and tabulated treatment wise and analyzed statistically using Software Package for social Sciences (SPSS version15.0). The data were subjected to one-way analysis of variance as per [Snedecor and Cochran \(1980\)](#). The difference within the means were estimated using Duncan's multiple range test ([Duncan, 1955](#)) by considering the differences at significant level ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Ammonia emission

There was no significant ( $P > 0.05$ ) difference in the litter ammonia emission between control and CC, however, a significant ( $P < 0.05$ ) reduction in was recorded in AS and ASCC (Figure 1a). AS was found to be highly effective in reducing the ammonia emission levels, either alone or in combination, with values of  $9.46 \pm 0.35$  (AS) and  $10.499 \pm 0.39$  (ASCC) compared to  $47.7 \pm 2.40$  and  $51.15 \pm 1.85$  ppm in CC and control during last week of trial. The reduction in ammonia volatilization from the

litter due to application of AS alone was by as much as 81.50 % and by combined application in ASCC group was 79.47 %. However, CC were effective in reducing the ammonia volatilization by only 6.74 % (Figure 1b).



**Figure 1. a)** Weekly ammonia emission in various groups and, **b)** percent reduction in ammonia emission compared to control in litter amended groups.

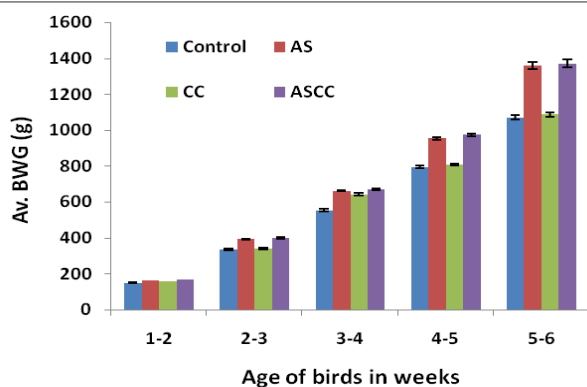
These results are in accordance to the earlier reports of and [Do et al. \(2005\)](#) and [Loch et al. \(2011\)](#) who found AS very effective in reducing the ammonia content. Similarly [Moore \(1995\)](#) observed that AS and ferrous sulfate reduced the ammonia volatilization from litter by as much as 99 and 58 % respectively. [Nagaraj et al. \(2007\)](#) also recorded reduction in ammonia emission by using litter amendment with sodium bisulphate. The litter amendments have been reported to reduce the litter moisture and subsequently the ammonia emission as wet litter has been associated with excessive ammonia production [Do et al. \(2005\)](#). Moreover, [Sahoo \(2016\)](#) found more cake formation in untreated litter as compared to the chemically treated litter. Caked litter negatively affects broiler chicken and contributes to more ammonia generation ([Kristensen et al., 2000](#); [Miles et al. 2004](#)). Further, as per [Terzich \(1997\)](#), litter pH has a decisive role in ammonia volatilization and the main ureolytic bacterium (*Bacillus pasteurii*) cannot grow in neutral pH but thrives in pH higher than 8.5 ([Terzich et al., 2000](#)). Since, the AS is acidic and CC is alkaline, it could be



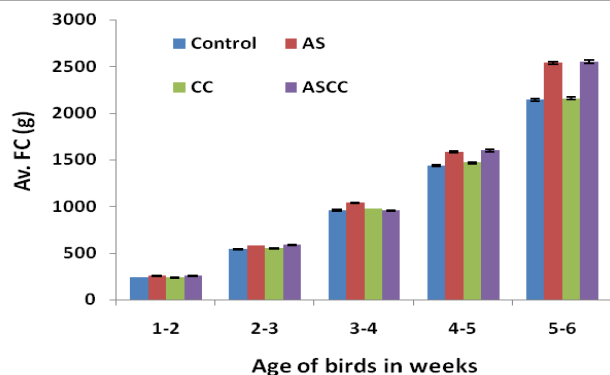
hypothesized that AS might have decreased the litter pH well below the neutral level, which would have hampered the growth of *Bacillus pasteurii*, thereby reducing the ammonia production drastically. It results in substantial amount of nitrogen in litter to remains in inorganic form, thus improving its value as a fertilizer as well (Moore, 1995).

### Performance of birds

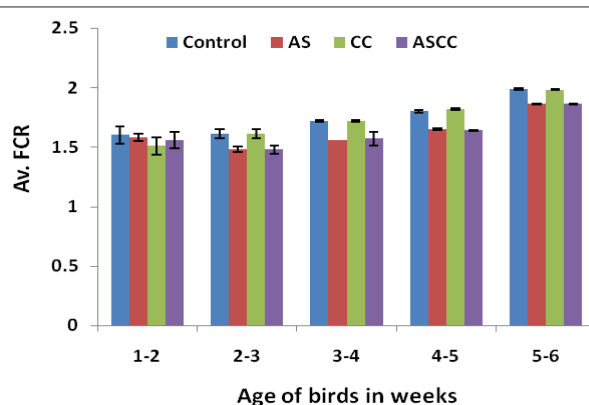
At the end of third week, there was a significant ( $P<0.05$ ) increase in the body weight gain (BWG) of chicks in AS and ASCC groups as compared to CC and control (Figure 2). A similar trend in the BWG was observed throughout the experiment with final BWG in of 1069.76 g in control, 1358.21 g in AS, 1086.66 g in CC and 1370 g in ASCC. Likewise, significantly ( $P<0.05$ ) highest FC (Figure 3) and improved FCR were observed in AS and ASCC groups in comparison to CC and control. At 6 weeks of age, improved FCR of 1.86 was observed in both AS and ASCC groups followed by 1.98 in CC and 1.99 in control (Figure 4). Thus, aluminum sulfate alone and in combination with calcium carbonate was found to be highly effective in improving the BWG and FCR of broiler chicken.



**Figure 2.** Average weekly body weight gain (g) in broiler chicken reared on chemically amended litter.



**Figure 3.** Average weekly feed consumption (g) broiler chicken reared on chemically amended litter.



**Figure 4.** Average weekly FCR of broiler chicken reared on chemically amended litter.

These results are in agreement with findings of Guo and Song (2009) who reported that broilers grown on AS treated litter had better weight gain in comparison with birds raised on untreated litter. However, Do et al. (2005) and Alkis and Celen (2009) found no significant difference in bird performance between the broilers reared on AS and combination of AS and calcium carbonate treated litter and control respectively. Birds in AS and ASCC groups consumed more feed and had better FCR than CC and control. Moore et al. (2000) also reported that birds on alum-treated litter had 4 % increased body weight and 3 % better FCR than in control. In the present study, the improvement in BWG of birds raised on chemically treated litter might be attributed to the reduction in ammonia production in AS group, which in turn has a role in alleviating the stress of birds (Kling and Quarles, 1974). Thus, reduction in ammonia emission in AS and ASCC groups might have improved the well-being of birds, resulting in better growth and FCR.

### Carcass characteristics

Among various slaughter traits, no significant ( $P>0.05$ ) difference was found among various groups as compared to control (Table 1). Moreover, there was no significant ( $P>0.05$ ) difference in the percent yield of giblets viz. gizzard, heart, liver and spleen; and weight of adrenal gland and bursa of fabricius among various treatment groups as compared to control (Table 2). These results are in contrast to Arias and Andkoutsos (2006) who reported improved dressed yield as a result on chemical treatment of litter with copper sulphate and attributed it to antibacterial activity of the chemical, thereby, improving the carcass quality of birds. Further, in the present study, no effect on yield of giblets and weight of vital organs was observed, thus confirming the reports of Younis et al. (2016) who used AS and copper sulphate in their study.

In conclusion, due to As's acidic nature, it was found to be highly effective in reducing the ammonia emission compared to CC. This in turn had a positive effect on the birds as indicated by improved performance in AS group.

Hence, the practice of acidic litter amendments rather than alkaline ones must be encouraged for beneficial broiler production.

**Table 1.** Effect of chemically amended litter on slaughter characteristics of broiler chicken up to 6 weeks of age (mean  $\pm$  S.E)

Parameter	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Pre-slaughter live weight (g)	1809 $\pm$ 127.3	1886 $\pm$ 51.07	1864 $\pm$ 128.8	1913 $\pm$ 71.08
De-feathered wt (g)	1646.6 $\pm$ 115.5	1713 $\pm$ 67.65	1683.3 $\pm$ 113.1	1723 $\pm$ 87.65
Dressed weight (g)	1296.6 $\pm$ 81.01	1360 $\pm$ 50.0	1316.6 $\pm$ 72.1	1370 $\pm$ 49.1
Dressing percentage	71.74 $\pm$ 0.57	72.07 $\pm$ 0.97	70.77 $\pm$ 1.43	71.77 $\pm$ 1.35

No significant difference was found among means of various treatments

**Table 2.** Effect of chemically amended litter on yield of giblets and weight of vital organs in broiler chicken up to 6 weeks of age (mean  $\pm$  S.E)

Parameter	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Gizzard weight, %	2.18 $\pm$ 0.19	2.18 $\pm$ 0.09	2.28 $\pm$ 0.29	2.19 $\pm$ 0.29
Heart weight, %	0.59 $\pm$ 0.08	0.58 $\pm$ 0.01	0.62 $\pm$ 0.04	0.57 $\pm$ 0.03
Liver weight, %	2.39 $\pm$ 0.14	2.44 $\pm$ 0.15	2.41 $\pm$ 0.26	2.46 $\pm$ 0.15
Spleen weight, %	0.21 $\pm$ 0.01	0.19 $\pm$ 0.02	0.22 $\pm$ 0.017	0.19 $\pm$ 0.01
Adrenal gland weight (mg)	66.8 $\pm$ 0.43	67.2 $\pm$ 0.32	67.0 $\pm$ 0.19	66.9 $\pm$ 0.38
Bursa of fabricius weight (g)	7.40 $\pm$ 0.29	7.39 $\pm$ 0.27	7.41 $\pm$ 0.23	7.38 $\pm$ 0.25

No significant difference was found among means of various treatments

#### Consent to publish

All persons gave their informed consent prior to their inclusion in the study.

#### Competing interests

The authors declare that they have no competing interests.

#### Author's contributions

This study is the part of M.V.Sc. Thesis of the first author AR, who carried out the research under the guidance of MTB. SA, AAK, SQ, MU and MAP helped during the trial, processing of samples and analysis of data. SA also helped in the technical writing of the article and its final revision. All authors have read and approved the final version of the manuscript.

#### REFERENCES

Alkis E and Celen MF (2009). Effects of alum treatment of two litter materials on growth performance of broiler chicken. *African Journal of Agricultural Research*, 4: 518-521.

Arias VJ, Andkoutsos EA (2006). Effects of Copper Source and Level on Intestinal Physiology and Growth of Broiler Chickens. *Poultry Science*, 85: 999-1007.

Bacharach U (1957). The aerobic breakdown of uric acid by certain pseudomonads. *Journal of General Microbiology*, 17: 1.

Do JC, Choi IH and Nahm KH (2005). Effects of chemically amended litter on broiler performances, atmospheric ammonia concentration and phosphorus solubility in litter. *Poultry Science*, 84: 679-686.

Đukić Stojčić M, Bjedov S, Žikić D, Perić L and Milošević N (2016). Effect of straw size and microbial amendment of litter on certain litter quality parameters, ammonia emission, and footpad dermatitis in broilers. *Archives Animal Breeding*, 59: 131-137.

Duncan DB (1955). Multiple range and multiple F-test. *Biometrics*, 11: 1-42.

Elliott HA and Collins (1982). Factors affecting ammonia release in broiler houses. *Transactions of the American Society of Agricultural Engineers*, 25: 413-424.

Galloway JN and Cowling EB (2002). Reactive nitrogen and the world: Two hundred years of change. *Ambio*, 31, 64-71.

Gates RS, Xin H, Casey KD, Liang Y and Wheeler EF (2005). Method for Measuring Ammonia Emissions from Poultry Houses. *Journal of Applied Poultry Research*, 14: 622-634.

Grimes J, Smith J and Williams C (2002). Some alternative litter materials used for growing broilers

- and turkeys. *World's Poultry Science Journal*, 58: 515-526.
- Guo M and Song W (2009). Nutrient value of alum-treated poultry litter for land application. *Poultry Science*, 88: 1782–1792.
- Hernandez R and Cazetta JO (2001). Simple and affordable method to determine ammonia released by the avian bed. *Revista Brasileira de Zootecnia*, 30: 824-829.
- Kling HF and Quarles CL (1974). Effect of atmospheric ammonia and the stress of infectious bronchitis vaccination on Leghorn males. *Poultry Science*, 61: 1052.
- Kristensen HH, Burgess LR, Demmers TGH and Wathes CM (2000). The preference laying hens for different concentrations of atmospheric ammonia. *Applied Animal Behavioural Science*, 68: 307-318.
- Loch FC, Oliveira MC, Silva D, Gonçalves BN, Faria BF and Menezes JFS (2011). Quality of poultry litter submitted to different treatments in five consecutive flocks. *Revista Brasileira de Zootecnia*, 40: 1025-1030.
- Lopes M, Roll V, Leite F, Dai Pra M, Xavier E, Heres T and Valente B (2013). Quicklime treatment and stirring of different poultry litter substrates for reducing pathogenic bacteria counts. *Poultry Science*, 92: 638-644.
- Miles DM, Branton SL and Lott BD (2004). Atmospheric ammonia is detrimental to the performance of modern commercial broilers. *Poultry Science*, 83: 501-509.
- Moore PA Jr (1995). Reducing ammonia volatilization from poultry litter with aluminum sulfate. In: *Proceedings of the Arkansas Nutrition Conference*, Fayetteville, AR. pp.84-93.
- Moore PA Jr, Daniel TC and Edwards DR (2000). Reducing phosphorus runoff and inhibiting ammonia loss from poultry manure with aluminum sulfate. *Journal of Environmental Quality*, 29: 37-49.
- Nagaraj M, Hess JB and Bilgili SF (2007). Evaluation of a feed-grade enzyme in broiler diets to reduce pododermatitis. *Journal of Applied Poultry Research*, 16: 52-61.
- Redding MR (2013). Bentonite can decrease ammonia volatilisation losses from poultry litter: laboratory studies. *Animal Production Science*, 53: 1115–1118.
- Ritz CW, Lowry A, Harper BD, Michael C and Jan P (2006). Evaluation of ferric sulfate as an ammonia control product in commercial broiler production. *Journal of Applied Poultry Research*, 15: 333-340.
- Salahuddin M, Wani SA, Pal MA, Ganaie TAS and Banday MT (2000). Carcass and meat yield characteristics of indigenous (desi) chicken in Kashmir. *SKUAST Journal of Research*, 2: 49-57.
- Sahoo SP (2016). Effect of chemically amended litter on litter quality and broiler performance in winter. *Journal of Applied Animal Research*, 1-5.
- Schefferle HE (1965). The decomposition of uric acid in built up poultry litter. *Journal of Applied Bacteriology*, 28: 412.
- Shah S, Westerman P and Parsons J (2006). Poultry litter amendments. North Carolina Cooperative Extension Service.
- Snedecor GW and Cochran WG (1980). *Statistical Methods*. 7<sup>th</sup> edition. Iowa State university press, Ames, USA.
- Terzich MAC (1997). Ammonia and pH level of the bed. *Conference of Science and Technology*, Sao Paulo, SP, Brazil, pp. 141-146.
- Terzich MAC, Pope MJ, Cherry TE and Hollinger J (2000). Survey of pathogens in poultry litter in the United States. *Applied Poultry Research* 9: 287-291.
- Younis MEM, Eman KAB, Ahmed HA and Elbestawy AR (2016). Broiler Performance, Carcass Characters and Litter Composition after management of fresh litter with two types of acidifier amendments. *International Journal of Agriculture Innovations and Research*, 4: 1050-56.

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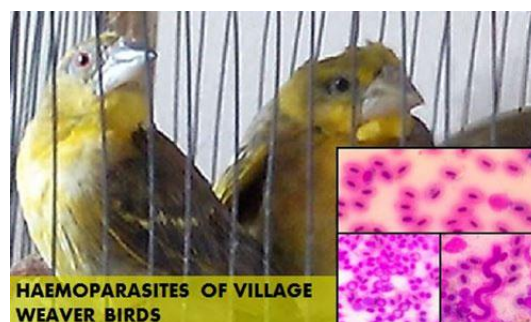
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<b>Decilitre</b>	dl	<b>Kilogram</b>	kg
<b>Milligram</b>	mg	<b>hours</b>	h
<b>Micrometer</b>	mm	<b>Minutes</b>	min
<b>Molar</b>	mol/L	<b>Mililitre</b>	ml
<b>Percent</b>	%		


Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (the Royal Society of Medicine London 1977). Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication".

#### Formulae, numbers and symbols:

1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter O, and between one (1) and the letter I.
2. Describe all symbols immediately after the equation in which they are first used.
3. For simple fractions, use the solidus (/), e.g. 10 /38.
4. Equations should be presented into parentheses on the right-hand side, in tandem.
5. Levels of statistical significance which can be used without further explanations are \*P < 0.05, \*\*P < 0.01, and \*\*\*P < 0.001
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. In chemical formulae, valence of ions should be given, e.g. Ca<sup>2+</sup> and CO<sub>3</sub><sup>2-</sup>, not as Ca<sup>++</sup> or CO<sub>3</sub>.
8. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
9. Greek letters should be explained in the margins with their names as follows: Αα - alpha, Ββ - beta, Γγ - gamma, Δδ - delta, Εε - epsilon, Ζζ - zeta, Ηη - eta, Θθ - theta, Ιι - iota, Κκ - kappa, Λλ - lambda, Μμ - mu, Νν - nu, Ξξ - xi, Οο - omicron, Ππ - pi, Ρρ - rho, Σσ - sigma, Ττ - tau, Υυ - ipsilon, Φφ - phi, Χχ - chi, Ψψ - psi, Ωω - omega.

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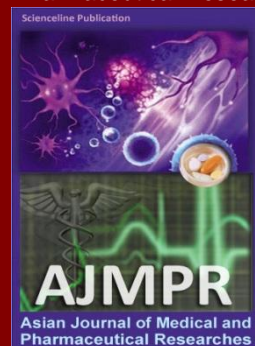
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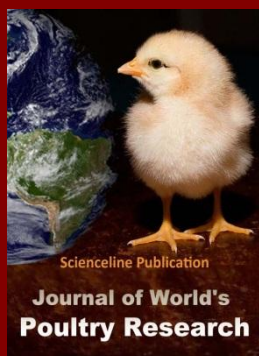
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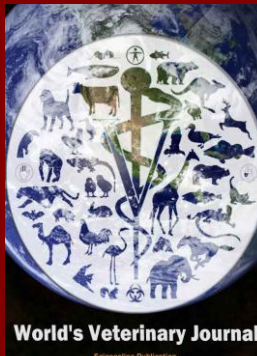
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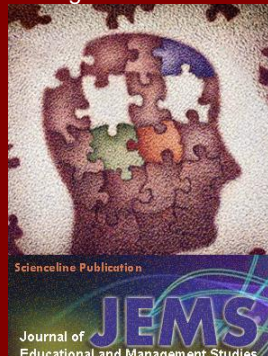
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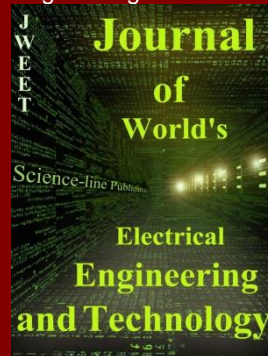
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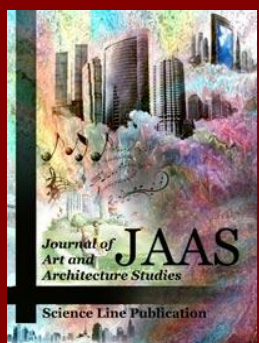
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