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# Effect of dietary humic acid via drinking water on the performance and egg quality of commercial layers

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## Abstract
This study was designed to investigate whether inclusions of humate into diets of hens during the late laying period increases egg production and improves egg quality. Hy-Line W-98 commercial White Leghorn (n=120), 50wk of age, were fed a control diet, 2.0, 4.0 or 6.0 mg of humic acid per kg live body weight for 65 d. Egg production was measured daily, egg weight and feed intake were measured biweekly. A sample of 30 eggs from each group was collected randomly to determine egg quality every 14d. The results showed that the laying hens that consumed drinking water with humic acid had greater ($P \leq 0.05$) egg production, egg weight and egg mass than the control. Similarly, feed conversion improved ($P \leq 0.05$) with the administration of humic acid in water. Yolk colour and egg grading from hens consuming humic acid water containing were significantly ($P \leq 0.05$) improved than those from the control group. Formic acid had no effect ($P \geq 0.05$) on albumin height and Haugh unit. In addition, the geometric means haemagglutination inhibition (HI) titres against Newcastle disease of birds consuming water containing humic acid were higher on all sampling days than those consuming water without acid. This study showed that humic acid at levels of 6.0 mg per kg live body weight positively influences production parameters including reduced mortality and feed conversion efficiency. However, there were no consistent effects on egg quality parameters.

## Keywords
Humic Acid, Egg Production, Egg Quality, Immunity

## 1. Introduction

Humic acids are naturally occurring decomposed organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains (MacCarthy, 2001). Using humic acid in animal nutrition has a very short history. Lenk and Benda (1989) and Griban *et al*. (1991) first used humic acid to improve the immune system of calves. Ku¨ hnert *et al*. (1991) used humic acid to treat digestive disorders and diarrhea of cats and dogs, respectively. Humic acid has also been used as analgesic and antimicrobial agent in veterinary practices in Europe (EMEA, 1999). Certain modified forms of humic acid have been shown to possess antiviral activities against herpes simplex and human immunodeficiency viruses (Klocking *et al*., 2002; Van Rensburg *et al*., 2002). Many experimental studies have shown humic acids to be largely nontoxic and nonteratogenic (EMEA, 1999).

In laying hen industry, egg quality and production are the most important economic facts. In this context age-related decline of egg production and shell quality are major factors affecting the profitability (Hakan *et al*., 2012). Prevent from these age-related problems, producers add mineral premixes or feed additives into layer diets (Ergin, 2009). Humic acid substances in laying hen diets like as feed additive is substantially new issue in this area. In recent years, it has been observed that humic acid included in the feed and water of poultry promote growth (Kocabag˘li *et al*., 2002; Rath *et al*., 2006; Mirnawati and Marlida, 2013). Humic acids improved egg production, egg weight (Yuruk *et al.,* 2004 and Kucukersan *et al.,* 2005), immune responses and electrolyte balance (Parks *et al.,* 1998). According to Shermer *et al.,* (1998), humic acid supplementation improved feed efficiency in poultry. There is some controversy regarding its effects on improvement in egg shell thickness on account of the reason that some previous studies had showed no effect on egg shell thickness (Yoruk *et al.,* 2004 and Hayirli *et al.,* 2005). To our knowledge, the effect of humic acid during the late laying period has not been tested. Therefore, the objective of this study was to investigate the effects of dietary humic acid on egg production, egg quality and immune responses when given to laying stock through drinking water during late laying phase.

## 2. Materials and Methods

### 2.1. Birds, Management and Diets

A total of 120 White Leghorn (Hyline W-98), 50-week old (30weeks in lay) were collected from Breeding & Incubation section, Poultry Research Institute, Rawalpindi, Pakistan. Hens were assigned to 4 treatment groups under the completely randomized design, so that there were 30 laying hens in each group. Each group was further divided into three sub-groups of 10 birds each (as replicates). The hens were supplemented with 0% (control group), 2.0, 4.0 or 6.0 mg of humic acid per kg live body weight, respectively through drinking water. The composition of basal diet is given in Table1. The basal diet was formulated to meet or exceed NRC (1994) recommendations for essential amino acids in laying feeding periods. Birds were reared on deep litter system. All hens were housed in an open shed with temperature maintained as close to 25.6°C as possible. The house was maintained ventilation and lighting (16L: 8D). During the experiment, layers were fed on an *ad libitum* basis at 8 a.m. and 3 p.m. and had free access to water. The trial was initiated on April 04, 2013, and terminated on June 07, 2013. The birds were vaccinated against Newcastle disease.

### 2.2. Humic Acid and Parameter Measured

Humic acid was purchased from Enrich feed (imported and marketed by Almuttahar, Shan Arcade Barkat Market, New Garden town, Lahore). It contained 70% humic acid and 12% sodium. Average daily water intake was recorded. Egg production was recorded daily at the same time and was calculated on a hen-day basis as follows: total number of eggs collected divided by total number of live hens per day in each group. Records of the feed intake were taken on bi-weekly basis. Birds were checked twice daily; weight of dead birds was used to adjust for feed consumption. Feed conversion was calculated as the ratio of grams of feed consumed to grams of egg mass.

Eggs were examined for interior and exterior quality. To determine egg quality characteristics, 30 eggs from each treatment group were used at 14-days interval of the experiment from a 2-d collection of eggs during the week. Egg mass was calculated as a factor of egg weight and hen-day egg production. Eggs were saved 2-d bi-weekly to measure egg weight. The egg weight, Haugh unit, yolk colour and egg grading were measured automatically by Egg Analyzer TM manufactured by Orka Food Technology Limited.

Diet was also analysed for proximate composition as described methods in AOAC (2011). All analysis and determinations were done in triplicate. Samples for proximate analysis were frozen until analyzed at the Feed Testing Laboratory, Poultry Research Institute, Rawalpindi.

***Table 1.*** *Composition of experimental basal diet*

| Ingredients | Composition (g/kg) |
|---|---|
| Corn | 480.00 |
| Rice Broken | 104.00 |
| Rice Polishing | 56.00 |
| Wheat Bran | 15.00 |
| Canola Meal | 70.00 |
| Rape Seed Meal | 30.00 |
| Guar Meal | 34.00 |
| Sunflower Meal | 30.00 |
| Soyabean Meal | 34.00 |
| Fish Meal | 30.00 |
| Molasses | 30.00 |
| Bone Meal | 15.00 |
| Marble Chips | 65.00 |
| Salt | 0.50 |
| L-Lysine | 1.00 |
| DL-Methionine | 0.70 |
| Vitamin Pre-mix[1] | 2.40 |
| Mineral Premix[2] | 2.40 |
| Total | 1000.00 |
| Calculate analysis | |
| M.E Kcal/Kg | 2740.00 |
| CP | 158.00 |
| C. Fat | 35.50 |
| C. Fibre | 47.80 |
| T. Ash | 93.80 |
| Calcium | 30.00 |
| Phosphorus(Available) | 3.60 |
| Lysine | 8.20 |
| Methionine | 3.90 |
| Methionine cystene | 6.30 |
| Sodium | 1.80 |
| NaCl | 3.80 |
| Lino | 13.50 |

[1]Provided the following per kilogram of diet: vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200 ICU; vitamin E (as dl-α-tocopheryl acetate), 8 IU; vitamin B12, 0.02 mg; riboflavin, 5.5 mg; d-calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B1 (thiamin mononitrate), 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg; vitamin K (menadione sodium bisulfate complex), 2 mg.

[2]Provided the following per kilogram of diet: manganese, 65 mg; iodine, 1 mg; iron, 55 mg; copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg.

### 2.3. Antibody Responses Against Newcastle Disease Virus

A La Sota vaccine was given to the hens via drinking water on day 1 of the experiment. Antibodies for Newcastle disease (ND) virus antigen in blood sera from 15 hens/treatment group were measured on days 1, 15, 30, 45 and 60 of the experiment by hemagglutination inhibition (HI) test as described by Thayer and Beard (1998). Serum was separated and processed for HI test at Disease Diagnostic Laboratory, Poultry Research Institute, Rawalpindi, Pakistan.

### 2.4. Statistical Analysis

Data were analyzed by SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL). The completely randomized design was applied. The differences between means were determined by ANOVA. When the differences were significant ($p<0.05$), Duncan's Multiple Range test was performed.

## 3. Results

### 3.1. Effect of Humic Acid on Layer Performance

Table 2 shows the effect of different levels of humic acid on the hen egg production, egg weight, egg mass, feed intake, feed conversion and mortality in hens. Administration of humic acid to the drinking water significantly increased ($P\leq0.05$) the egg production, egg weight, egg mass, feed conversion and yolk colour than the control group. Egg production (%) in the hens consuming water with 0, 2.0, 4.0 or 6.0 mg/kg live weight was approximately 64.80, 65.64, 69.80 and 74.07%, respectively. There was approximately 2–3 g increase in the egg weights from laying hens consuming drinking water with different levels of humic acid compared with the control group. Egg mass was also increased ($P\leq0.05$) progressively with increasing level of humic acid. Similarly, feed conversion improved ($P\leq0.05$) by 0.13, 0.33 and 0.42 points with the administration of humic acid in water, respectively than the control. There was no significant ($P > 0.05$) effect on feed intake.

The mortality of the birds in the present trial was in the expected range and was not influenced by the administration of humic acid to the drinking water. No changes in the health status of chickens were seen during the entire experimental period. The cause of death of chickens in all groups was the sudden death syndrome.

### 3.2. Egg Quality

In this study, there were no adverse effects of humic acid at any levels on the quality of the eggs (Table 2). Height of albumin and Haugh unit score did not differ significantly ($P > 0.05$) among the laying hens at all levels of humic acid groups. However, yolk colour and egg grading were improved ($P \leq 0.05$) in the groups consuming water with humic acid as compared with control group.

***Table 2.*** *Effect of humic acid supplementation on egg production, egg weight, egg mass, feed intake, feed conversion, mortality and egg quality (height of albumin, yolk color, egg grading and Haugh unit)*

| Parameter | Humic acid levels (mg/kg live weight) | | | |
|---|---|---|---|---|
| | Control | 2.0 | 4.0 | 6.0 |
| Egg production (%) | $64.80\pm1.48^{b}$ | $65.64\pm1.56^{b}$ | $69.80\pm1.43^{ab}$ | $74.07\pm1.51^{a}$ |
| Egg weight (g) | $51.45\pm0.73^{b}$ | $54.01\pm1.23^{a}$ | $54.38\pm0.34^{a}$ | $54.56\pm0.78^{a}$ |
| Egg mass (g/d/hen)† | $33.33^{b}$ | $35.45^{ab}$ | $37.96^{ab}$ | $40.41^{a}$ |
| Feed intake (g) | 93.38±1.08 | 94.71±0.84 | 93.87±0.81 | 96.29±1.71 |
| Feed conversion (g feed:g egg mass) | $2.80\pm0.01^{a}$ | $2.67\pm0.03^{ab}$ | $2.47\pm0.01^{b}$ | $2.38\pm0.06^{bc}$ |
| Mortality (%) | $1.0^{a}$ | $0.5^{b}$ | $0.5^{b}$ | $0.5^{b}$ |
| Height of Albumin (mm) | 4.11±0.25 | 4.38±0.14 | 3.64±0.27 | 4.20±0.27 |
| *Yolk color | $4.71\pm0.26^{b}$ | $5.22\pm0.25^{ab}$ | $5.05\pm0.31^{ab}$ | $5.54\pm0.30^{a}$ |
| Egg grading | B | A | A | A |
| Haugh unit | 58.78±2.91 | 58.89±4.03 | 62.88±3.03 | 64.42±1.40 |

Different superscripts show level of significance at $P \leq 0.05$
*1 = maximum pale color while 10 = maximum dark yellow color
†Egg mass = (egg production x egg weight)/100.

### 3.3. Immunity

***Table 3.*** *Effects of humic acid administration in the drinking water on anti-ND haemagglutination-inhibition titres*

| Humic acid (mg/kg live weight) | Geometric Means Hi Titers | | | | |
|---|---|---|---|---|---|
| | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 |
| Control | 3.17 | 3.97 | 4.95 | 6.10 | 6.50 |
| 2.0 | 3.00 | 5.00 | 6.00 | 8.00 | 9.20 |
| 4.0 | 3.96 | 5.10 | 6.30 | 8.65 | 9.56 |
| 6.0 | 3.60 | 5.62 | 6.85 | 9.30 | 9.90 |

The geometric means HI titres of hens consuming water with humic acid were higher from 30 to 60 days than that of control (Table 3). In the current study, antibody titre against ND in layers increased by increasing the levels of humic acid. These results showed that humic acid administration in water tended to increase immunity.

## 4. Discussion

According to previous work, different doses of humic acid showed improvement in layer performance. Kucukersan *et al.* (2005) reported that the dietary supplementation of humic acid at doses of 30 and 60 g/t feed can be used to improve egg production, egg weight and feed efficiency. Similarly, another study showed that egg production and egg mass increased with supplementation of 30 or 90ppm of humic acid in layer diet (Ergin *et al*., 2009). Alteration in nutrient partitioning could be associated with increased egg production in hens receiving supplemental humate (Hayirli *et al*., 2005). A lack of effects of the different level of humic acid through drinking water on feed intake could be related to the completion of the growing process at this age. In relation to this, there could be less variation in gastrointestinal tract capacity of older hens. Yörük *et al*. (2004) observed that feed conversion efficiency (weight of feed/weight of eggs) in laying hens decreased linearly with increasing concentrations of supplemental humate. Similar trend was observed in the current study. Like current study, some researchers found that mortality rate in broilers and laying hens was not affected by the supplementation of humic acid (Yörük *et al*., 2004; Karaoglu *et al*., 2004; Özçelik and Yalçın, 2004 and Islam *et al*., 2008).

Little is known about the mechanism by which humate supplementation enhances the life span and improves production efficiency. However, available data consistently suggest that humate supplementation may benefit poultry production. The positive effect of humic acid on the above parameters might be due to its ability to influence in particular the metabolism of protein carbohydrates of microbes by catalytic means. This leads to a direct devastating of bacterial cells or virus particles which should result in improved bird's performance (Huck *et al*., 1991).

The detoxifying benefits of humic acid in the soil cover the full spectrum of accumulated toxins associated with chemical farming. When humic acid is added to the diet, heavy metals, nitrates, fluoride, organophosphates, carbaryl and chloride organic insecticides can be adsorbed and excreted (Islam *et al*., 2005). The humic acid can form a protective film on the mucus epithelium of the gastrointestinal tract against infections and toxins, thus ensuring an improved utilization of nutrients in animal feed (Islam *et al*., 2005).

Previous studies showed inconsistent results with supplementation of humic acid on egg quality traits. Like current study, Macit *et al*. (2009) reported that yolk colour improved with supplementation of different levels of humate (0.10, 0.15, 0.20, 0.25, 0.30 and 0.35wt% humate ) in layer diets. In contrast of above studies, Dobrzańsk *et al*. (2009) reported that yolk colour did not improve with supplementation of humic acid (4.5 g/hen/day). Yolk colour is an important to consumers. Recent surveys in a number of different European countries (France, Germany, Italy, UK, Spain, Poland and Greece) have confirmed that yolk colour is one of the main parameters by which the quality of eggs is judged (Beardsworth and Hernandez, 2004).

There were no significant differences between the groups in height of albumin and Haugh unit in the present study. These results are concurred with the findings of Kucukersan *et al*. (2005), who reported that albumen index and Haugh unit were not closely related to the addition of humic acid to layer hen diets. Similarly, Yörük *et al*. (2004) reported that supplementation of humate during laying period did not improve egg quality. However, some contradictory results have also been reported by other researchers, such as Macit *et al*. (2009), who reported that humic acid exerted a positive effect on the egg quality particularly albumin index and Haugh unit. The incompatible results observed in different studies may be attributable principally to the composition of different humic substances preparations, and addition levels, as well as the different animal species and ages used in different studies.

Earlier studies showed that supplementation of humate improved the immunity of birds. Parks (1998), who demonstrated that humate improved cell-mediated immunity of turkeys fed low crude protein diets. Similarly, Mehdi and Hasan (2012) reported that supplemental humic acid (0.1to 0.3%) in broiler diets improved antibody titers against ND virus. Rath *et al*. (2006) reported that the relative weights of the bursa of fabricius increased in birds given 0.25% humate suggesting a possible immunostimulatory effect that has been considered to be an effect of humate. Humic acid may exert a beneficial effect on immune systems of birds. The action mechanism in humic acid is related with their potential to form complex saccharides in the body, which function as modulators of intercellular interaction. These maintain the balance of the immune system activity, and prevent potential inadequate responses (Riede *et al*., 1991). Humic acid stimulates the resistance forces of the body, and result in an increase in the phagocytic activity. Cetin *et al*. (2011) reported that supplementation of humic acid (0.15%) in laying hens resulted in significant increases in the lymphocyte counts via the increased production of IL-2 and the expression of IL-2 receptors on lymphocye which resulted in the enhancement of the activity of IL-2 producing cells. Terratol (2002) also reported that humic acid may stimulate the production of glycoproteins, which can regulate the immune system via the maintenance of the balance of killer and T cells.

## 5. Conclusion

It can be concluded that dietary humic acid (6.0 mg/kg live weight) had significant effects on production parameters including reduced mortality and feed conversion efficiency and increased egg production. However, there were no consistent effects on egg quality parameters. Therefore, supplementation of humate at higher level (6.0 mg/kg live weight) may extend the profitability of a layer flock.

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