Effects of humic acid on some hematological parameters, total antioxidant capacity and laying performance in Japanese quails

Einfluss von Huminsäure auf einige Blutparameter, auf die gesamte antioxidative Kapazität und auf die Legeleistung von Japanischen Wachteln

H. Ipek¹, M. Avci², M. Iriadam¹, O. Kaplan² and N. Denek²


Introduction

Antimicrobial feed additives are worldwide used in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, by decreasing feed expenditure per gain and by diminishing the risk of disease (Islam et al., 2005). But subtherapeutic use of antibiotic in poultry feeds has become undesirable because of the residuals in animal products, such as meat and eggs, and the development of antibiotic resistant bacterial populations in human and veterinary therapy. So, in the European Union, the use of antibiotics as growth-promoting agents for poultry has been banned (Mutas et al., 2006) and consumer pressure will likely lead to voluntary withdrawal of the drugs from use in other countries (Guban et al., 2006).

In association with the banned use of antibiotic stimulants in animal nutrition, prebiotic, probiotic and humic acid (HA) have been alternatively used besides antibiotics to minimize health problems and potential losses and to increase performance in poultry flocks (Yoruk et al., 2004; Kucukersan et al., 2005; Trckova et al., 2006). The use of humic acid to replace antibiotics in poultry has gained widespread interest (Mutas et al., 2006).

HA shows antibacterial, antiviral, antithyroideal and anti-inflammatory effects in animals, and improves the immune system. It also has positive effect on live functioning, ultimately reduces mortality and increases growth in poultry (Islam et al., 2005). HA has demonstrated a strong affinity to bind various substances, such as heavy metals (Madronova et al., 2001), mutagens (Sato et al., 1987), minerals (Elfarissi and Pefferkorn, 2000), bacteria (Riede et al., 1991), and aflatoxins (Van Rensburg et al., 2006). These physicochemical properties of HA may also be responsible for some of the effects occurring in tissues, including the elimination of heavy metals (Madronova et al., 2001), desmutagenic effects (extracellular interception of mutagens) (Sato et al., 1987) and antibacterial effects (Riede et al., 1991).

There are different effects of HA on oxidative stress in human and animals. Adington and Schauss, (1999) have proposed that HA administered at physiological levels could play a protective role during myocardial reperfusion by exhibiting antioxidant activity. In the same report, it was remarked that HA may have the ability, as an antioxidant, to limit the potential formation of oxyradicals produced during tissue injury which occurs with ischemia and reperfusion. It has also been proposed that HA plays an effective role on some blood parameters (Rathi et al., 2006; Banaszkiewicz and Drobnik, 1994; Cetin et al., 2006).

It has been indicated that HA had differentiated effects upon trace elements in rats. Plasma iron levels were hardly affected, while copper and zinc levels were initially suppressed with a tendency for recovery after 60 days (Islam et al., 2005). After humate feeding, increased levels of some essential minerals (such as Ca, Al and Fe) in serum, liver and muscles were recorded by Stepchenko et al., (1991).

Limited studies have been conducted to determine the effect of HA on some blood parameters, egg production and total antioxidant capacity in poultry. To our knowledge, the effect of HA on some blood parameters and total antioxidant capacity in quails have not been tested. Therefore, the objectives of this study were to investigate the effects of supplementation of HA to the diet on some blood parameters, egg production (EP), feed consumption (FC), feed efficiency (FE), egg weight (EW) and total antioxidant capacity (TAC) in Japanese quails.

Materials and Methods

A total of 140 (nine weeks old) female Japanese quails (Coturnix coturnix japonica) at the beginning of the laying period were used. The quails were randomly assigned to one control and three experimental groups, comprising five replicates of 7 birds each (each group having 35 quails). The birds were housed in an 8 m x 7 m quail house equipped with 4 cage blocks with wire mesh floor. Each caging unit contained 5 subcages (60X20X20 cm), 7 birds per subcage. Each cage compartment was equipped with a nipple drinker and a trough-type feeder.

Quails were fed a basal diet (control group) and or the basal diet supplemented with either 360 (group I), 480 (group II) and 600 (group III) mg/kg of humic acid, respectively. HA (sodium salt) was obtained from Biyotar Organic Agriculture and Chemical Company (Ankara, Turkey).

Small amounts of the basal diet were first mixed with the respective amount of HA as a small batch and then with...
a larger amount of the basal diet until total amounts of the respective diets were homogeneously mixed. Feed were prepared weekly. Chemical compositions of the diets were analyzed using the international procedures of AOAC (2000). The formula of calculating ME is \( ME, \text{kcal/kg} = 53 + 38 \times \text{CP, %} + 2.25 \times \text{EE, %} + 1.1 \times \text{starch, %} + 1.05 \times \text{sugar, %} \) (CARPENTER and C LEGG, 1956). Ingredients and chemical composition of the basal diet fed to Japanese quails are shown in Table 1.

The poultry house was lit 24 h/day. The diets were offered ad libitum throughout the experimental period lasting for five weeks. FC, FE, EW and EP data were recorded in weekly intervals.

**Blood Sampling Protocol**

At the end of five weeks, the ten animals in each group were decapitated and blood samples were collected into heparinized tubes in the fasting state lasting four hours. Blood samples were taken for hematological analyses. The remaining sample was centrifuged at 1400 x g for 10 min for plasma separation. Plasma samples were stored at -80°C until TAC analysis. Hematocrit value (PCV) was measured by microhematocrit method. The red blood cell (RBC) and white blood cell (WBC) counts were determined by a hemocytometer method using Natt-Herrick solution (KONUK, 1981). Differential counts were performed on 10 of the birds using Wright-Geimsa–stained thin blood smears. Heterophil to lymphocyte ratio was calculated from counts of 200 WBC per quail (WORK et al., 2001). Hemoglobin concentration (Hb) was determined by spectrophotometry (540 nm) after the blood was mixed with Drabkin solution (FARFANKS and KLEE, 1999). Plasma iron concentration was measured by automated chemistry analyzer (Aerose, Abbott, USA) using commercial kits (Abbott). Copper concentration was determined by a spectra AA 250 plus Zeeman Atomic Absorption Spectrometer (Varian, Australia) with a deuterium background correction.

**Measurement of Total Antioxidant Capacity**

TAC of serum was determined using a novel automated measurement method, developed by EREL, (2004). In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, a standardized solution Fe+++-o-dianisidine complex reacts with a standardized solution of hydrogen peroxide by Fenton-type reaction, producing hydroxyl radical. These potent reactive oxygen species oxidize the reduced colorless o-dianisidine molecules to yellow-brown colored dianisidyl radicals at low pH. The oxidation reactions progress among dianisidyl radicals and further oxidation reactions develop. The color formation is increased with further oxidation reactions. Antioxidants in the sample suppress the oxidation reactions and color formation. Lower value of TAC indicates higher oxidative stress. Accuracy of the method is >97%. The results are expressed as mmol Trolox equiv/l (EREL, 2004).

**Statistical analysis**

Data were statistically analyzed by a one-way ANOVA and the means were compared by Duncan’s multiple-range test (STEEL and TORRIE, 1981).

Table 1. Composition of experimental diets (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>59.125</td>
<td>58.80</td>
<td>58.725</td>
<td>58.60</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>28.00</td>
<td>28.00</td>
<td>28.00</td>
<td>28.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>2.00</td>
<td>2.10</td>
<td>2.15</td>
<td>2.10</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>5.40</td>
<td>5.40</td>
<td>5.40</td>
<td>5.40</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Vitamin mineral premixa</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>DL-Methionin</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>Humic acid (16%)</td>
<td>----</td>
<td>0.225</td>
<td>0.300</td>
<td>0.375</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Chemical analysis, drymatter (DM) basis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>19.98</td>
<td>20.00</td>
<td>20.01</td>
<td>20.03</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.51</td>
<td>2.51</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>0.34</td>
<td>0.35</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>Calculated values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME (Kcal/Kg)</td>
<td>2900</td>
<td>2893</td>
<td>2890</td>
<td>2890</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.11</td>
<td>1.11</td>
<td>1.11</td>
<td>1.11</td>
</tr>
<tr>
<td>Methionine+cystine</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
</tbody>
</table>

*Vitamin premix provided the following per kg diet: Vitamin A, 12500 IU; Vitamin D3, 1500 IU; Vitamin E, 31.25 mg; Vitamin K3, 3.75 mg; Vitamin B1, 2.5 mg; Vitamin B2, 7.5 mg; Niacin 25 mg; Cal. D-pantothenate 10 mg; Vitamin B6, 5mg; Vitamin B12, 0.019 mg; Folic acid 1 mg; Choline chloride 250 mg; Mn 100 mg; Fe 75 mg; Zn 75 mg; Cu 6.25 mg; Co 0.25 mg; I, 1.25 mg; Se 0.19 mg.
Results

Dietary HA supplementation affected both TAC and some blood parameters. The effects of supplementation of HA on some blood parameters and TAC are shown in Table 2.

While Hb concentration was significantly (p<0.05) higher in all the experimental groups than in the control group, RBC was only (p<0.05) significantly higher in group I, compared with the control group and PCV was (p<0.05) significantly higher in group II and group III. No significant differences were found in WBC, levels of plasma copper, heterophil and lymphocyte percentage between the experimental and the control groups (p>0.05). There was a gradually increase in the levels of plasma iron, which was significant (p<0.05) in group III. TAC was significantly (p<0.05) lower in group III than in the other groups. Similarly, heterophil to lymphocyte ratio was significantly (p<0.05) lower in group I when compared with the control group.

Regarding laying performance parameters such as FC, EW, EP and FE, no significant differences were found in all the groups.

Discussion and conclusion

This study was designed to evaluate the effects of HA upon EP, FC, FE, TAC and some blood parameters in Japanese quails. There was a close inverse correlation between TAC and high levels of HA. HA was positively correlated to plasma copper, iron and zinc levels. Some blood parameters and TAC are shown in Table 2.

Table 2. Effects of humic acid on TAC and some hematological parameters (Mean ± Standard Error, n=10)

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>Group I (n=10)</th>
<th>Group II (n=10)</th>
<th>Group III (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10^6 mm^-3)</td>
<td>3.25 ± 0.09b</td>
<td>3.50 ± 0.10ab</td>
<td>3.55 ± 0.09a</td>
<td>3.75 ± 0.09a</td>
<td>**</td>
</tr>
<tr>
<td>WBC (×10^6 mm^-3)</td>
<td>37.50 ± 3.41</td>
<td>34.80 ± 2.33</td>
<td>35.80 ± 2.81</td>
<td>32.20 ± 1.27</td>
<td>ns</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.80 ± 0.37b</td>
<td>12.25 ± 0.49a</td>
<td>12.50 ± 0.24a</td>
<td>13.30 ± 0.29a</td>
<td>**</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>40.70 ± 1.29c</td>
<td>44.80 ± 1.78bc</td>
<td>49.30 ± 1.72ab</td>
<td>51.00 ± 2.42a</td>
<td>**</td>
</tr>
<tr>
<td>Heterophil (%)</td>
<td>51.20 ± 1.62</td>
<td>46.60 ± 1.65</td>
<td>48.30 ± 1.22</td>
<td>48.30 ± 2.33</td>
<td>ns</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>39.00 ± 1.21</td>
<td>45.80 ± 1.74</td>
<td>43.50 ± 2.31</td>
<td>44.30 ± 2.04</td>
<td>ns</td>
</tr>
<tr>
<td>Heterophil/Lymphocyte</td>
<td>1.33 ± 0.07a</td>
<td>1.03 ± 0.05b</td>
<td>1.14 ± 0.07ab</td>
<td>1.12 ± 0.08b</td>
<td>*</td>
</tr>
<tr>
<td>Cu µg/dl</td>
<td>22.79 ± 2.22</td>
<td>22.69 ± 1.35</td>
<td>20.45 ± 1.22</td>
<td>20.15 ± 1.31</td>
<td></td>
</tr>
<tr>
<td>Fe µg/dl</td>
<td>93.92 ± 6.73 b</td>
<td>97.48 ± 6.19 b</td>
<td>107.07 ± 7.30 ab</td>
<td>121.22 ± 6.20 +</td>
<td>*</td>
</tr>
<tr>
<td>TAC mmol Trolox equiv/l.</td>
<td>0.62 ± 0.03a</td>
<td>0.67 ± 0.05a</td>
<td>0.59 ± 0.06a</td>
<td>0.46 ± 0.02b</td>
<td>*</td>
</tr>
</tbody>
</table>

a-c: Values bearing different superscripts in the same line indicates significant difference.
ns P>0.05; * P<0.05; ** P< 0.01

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As HA can act as a strong metal chelator, it is also possible that HA chelates extracellular iron ions and carries iron into cells, which may be explained as a reason of this increase. Therefore, HA enhanced the accumulation of iron in endothelial cells and induced an elevation in intracellular chelatable iron, leading to the ROS generation and lipid peroxidation when it is administered for long time at high levels (Gau et al., 2001; Addington and Schauss, 1999).

Antioxidative effects of different antioxidant components of plasma are additive and the measurement of them separately is not practical. Therefore, the measurement of total antioxidant capacity (TAC) reflects accurately the antioxidative status of the organism (Erel, 2004).

No significant differences were found in FC, FE, EW and EP between the experimental and the control groups (p>0.05).

Dietary HA supplementation increased RBC, Hb, PCV and plasma iron. Therefore, HA may treat iron deficiency and anemia. However, these results, obtained in the third experimental group administered with high levels of HA, showed that at high concentrations of HA oxidative stress is increased. Whereas, the higher TAC value obtained in group I which received a low level of HA indicates that supplementation of low levels of HA to diets did not enhance oxidative stress.

In conclusion, results demonstrate that HA has increased RBC, Hb, PCV and plasma iron levels and did not have any effect on WBC, plasma copper, EP, FC and FE. While high concentrations of HA decreased TAC, its lower levels did not effect TAC. Therefore, high levels of HA should not be supplemented because of increasing oxidative stress.

Summary

The objectives of this study were to investigate the effects of supplementation of humic acid (HA) on some blood parameters, egg production (EP), feed consumption (FC), and total antioxidant capacity (TAC). Therefore, high levels of HA should not be supplemented because of increasing oxidative stress.

Key words

Nutrition, humic acid, blood parameters, TAC, quail

Zusammenfassung

Einfluss von Huminsäure auf einige Blutparameter, auf die gesamte antioxidative Kapazität und auf die Legeleistung von Japanischen Wachteln

Das Ziel dieser Arbeit war, den Einfluss der Zugabe von Huminsäure (HA) zum Futter auf einige Blutparameter, Legeleistung (EP), Futteraufnahme (FC), Futterverwertung (FE), Eigewicht (EW) und die gesamte antioxidative Kapazität (TAC) bei Japanischen Wachteln zu untersuchen. Für die Untersuchung wurden 140 Japanische Wachteln im Alter von 9 Wochen verwendet. Die Wachteln wurden mit einem Futter ohne HA (Kontrollgruppe) bzw. mit 360 (Gruppe I), 480 (Gruppe II) oder 600 (Gruppe III) mg HA/kg Futter gefüttert. Blutproben wurden in Röhrenchen mit Heparin gesammelt und neben verschiedenen Blutparametern auch die Eisen- und Kupferkonzentrationen im Plasma bestimmt. Die TAC-Gehalte im Serum wurden mit einer neuen automatisierten Methode analysiert.

Der Zusatz von Huminsäure zum Futter hat sowohl die TAC-Werte als auch einige Blutparameter beeinflusst. Die Hämoglobinkonzentration (Hb) war bei allen Testgruppen und die Anzahl der roten Blutkörperchen (RBC) war nur bei der Gruppe I höher als bei der Kontrollgruppe (p<0.05). Der Hämakritiwert (PCV) war bei den Gruppen II und III signifikant höher (p<0.05). In Abhängigkeit von der Zulage an Huminsäure wurde eine stetige Zunahme der Plasma-Eisenkonzentration registriert, die für Gruppe III signifikant war (p<0.05). TAC war in Gruppe III signifikant niedriger als in den anderen Gruppen (p<0.05). Das Verhältnis von Heterophilen zu Lymphozyten war in Gruppe I im Vergleich zur Kontrollgruppe signifikant geringer (p<0.05). Zwischen den Gruppen wurden keinen Unterschiede hinsichtlich der EP, FC, FE, EW und Leukozytenzahl (LC) gefunden.


Stichworte

Fütterung, Huminsäure, Blutparameter, TAK, Wachtel

References


KONUK, T., 1981: Practical Physiology, University of Ankara Faculty of Veterinary Medicine Press, Ankara.


Correspondence: Hudai Ipek PhD, Harran University, Faculty of Veterinary Medicine, Department of Physiology, Campus of Yenisehir, Sanliurfa, Turkey; e-mail: hudaiipek@harran.edu.tr, hudaiipek@hotmail.com, hudaiipek@gmail.com


