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*Full Length Research Paper*

# Herbal additives and organic acids as antibiotic alternatives in broiler chickens diet for organic production

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**This experiment was conducted to compare the influence of antibiotic, organic acids and herbal additives on broiler chickens. Treatments included: (1) basal diet (2) mixture of organic acids (3) shallot (4) yarrow (5) mixture of shallot and yarrow and (6) antibiotic. Significantly, increased feed intake (FI) was found by all treatments as compared to the control group. Significant higher body weight gain (BWG) and better feed conversion ratio (FCR) were observed by shallot, shallot and yarrow mixture and antibiotic treatments than other treatments. Breast and tight yields of control and organic acids treatments were significantly increased than other treatments at day 21. Significantly longer intestine was also shown by shallot and yarrow treatments as compared to the control group at day 42. Height and width of villus and crypt depth of bird fed with shallot diet were significantly greater than others at day 21, and these followed similar pattern at day 42. Significant increase in lactic acid bacteria counts in ileum and cecum of broiler chicken was shown by all treatments as compared to the control at day 21. In comparison to the control, all treatments significantly decreased *Enterobacteriaceae* counts in ileum and cecum of broiler chicken at 21 and 42 days of age. Moreover, protein, DNA and RNA contents were not affected by all treatments.**

**Key words:** Broiler, antibiotic, organic acid, herbal.

## INTRODUCTION

Recently, antibiotic growth promoter in poultry industry has been banned because harmful effects on human health was observed by development of microbial resistance to these products (Botsoglu and Fletouris, 2001; Williams and Losa, 2001; McCarteney, 2002). Several alternatives to antibiotic growth promoters have been proposed, such as organic acids, probiotics, herbs and herbal products. Organic acids and medicinal plants as natural feed additives are recently used in poultry diet to enhance the performance and the immune response of birds. Many of the organic acids with beneficial effects on animal performance are also known to be effective food and feed preservatives (Dibner and Buttin, 2002). Supplementation of these additives in the diet of broilers enhanced nutrient utilization, growth and feed efficiency (Denil et al., 2003).

Persian shallot or *Allium ascalonicom* is a species of the family *Liliaceae*, and a native plant in Iran (Harris et al., 2001; Taran et al., 2006). Traditional medicine uses genus *Allium* plants for treatment of infectious diseases such as bacterial, fungal, viral, protozoal and helminthic diseases (Harris et al., 2001; Taran et al., 2006). Also, yarrow (*Achillea millefolium*), belonging to the *Asteraceae* family, is used widely in many parts of the world (Chevallier, 1996). For many centuries, various species of genus *Achillea* have been used as folk medicines for the curing of various diseases (Saeidnia et al., 2005). Yarrow is used for disorders of the respiratory, digestive, hepatobiliary, cardiovascular, urinary and reproductive systems (Blumenthal et al., 1998). Extracts of yarrow have demonstrated antimicrobial activity against a wide range of bacteria (Barel et al., 1991). However, no studies have evaluated the effect of shallot and yarrow in broiler nutrition.

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**Table 1.** Ingredient's composition (as percent of dry matter) and calculated analysis of the basal diets.

| Ingredient | Starter (0 to 21 day) | | Grower (22 to 42 day) | |
|---|---|---|---|---|
| | Control | X[6] | Control | X |
| Corn | 47 | 45.28 | 57 | 55.8 |
| Soybean meal | 38.1 | 38.3 | 29.15 | 29.9 |
| Wheat | 8 | 8 | 9.5 | 8.15 |
| Soybean oil | 3.1 | 3.6 | 1 | 1.8 |
| DCP | 1.6 | 1.6 | 1.3 | 1.3 |
| Oyster shell meal | 1.3 | 1.3 | 1.2 | 1.2 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 |
| Vit and min premix[1] | 0.5 | 0.5 | 0.5 | 0.5 |
| DL- Methionine | 0.1 | 0.1 | 0.04 | 0.04 |
| Additive[2] | 0 | 1 | 0 | 1 |
| Total | 100 | 100 | 100 | 100 |
| | | | | |
| **Nutrient content** | | | | |
| ME[3] (Kcal/kg) | 3000 | 3000 | 3000 | 3000 |
| CP[4] (%) | 21.56 | 21.56 | 18.75 | 18.75 |
| Ca (%) | 0.93 | 0.93 | 0.84 | 0.84 |
| AP[5] | 0.42 | 0.42 | 0.33 | 0.33 |
| Met | 0.47 | 0.47 | 0.36 | 0.36 |
| Met + Cys | 0.84 | 0.84 | 0.67 | 0.67 |

[1]Vitamin and mineral provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 20 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, mg; choline chloride, 200000 mg, manganese, 40000 mg, iron, 20000 mg; zinc, 40000 mg, copper, 4000mg; iodine, 400 mg; selenium, 80 mg; [2] organic acid mixture, shallot, yarrow, mixture of shallot and yarrow or antibiotic; [3] Metabolisable energy; [4] crude protein; [5] available phosphorous; [6] basal diet supplemented with different additives.

The purpose of this study was therefore to investigate the effect of organic acids and herbal additives as antibiotic alternatives on performance, carcass characteristics, small intestine villus, microbial population, protein and nucleic acids content of broiler chickens.

## MATERIALS AND METHODS

All experimental procedures were approved by the Animal Research Ethics Committee of the Bu-Ali Sina University, Hamedan, Iran.

### Bird management and diets

200 88-day old unsexed broiler chicks (Ross 308) were randomly assigned to 5 treatments with 4 replicates and 12 chicks per each replicate pen at a 42-day study. Birds were treated as follows: (1) basal diet (corn-soybean meal); (2) basal diet supplemented with 1 kg/ton mixture of organic acids, (3) basal diet supplemented with 1 kg/ton shallot, (4) basal diet supplemented with 1 kg/ton yarrow, (5) basal diet supplemented with 1 kg/ton mixture of shallot and yarrow (1:1 ratio), and (6) basal diet supplemented with 1 kg/ton antibiotic. It is important to note that the used antibiotic was virginiamycin and organic acids mixture included malic, formic, citric, ortophosphoric, lactic and acetic acids. A basal diet was formulated as control according to NRC recommendations (NRC, 1994) for starter (0 to 21 days), and grower (22 to 42 days) periods (Table 1). All diets were provided as a coarse mash. Birds were raised on floor pens and received feed and water *ad libitum*. Light was also provided continuously (23 h light : 1 h dark) throughout the experimental period and the initial room temperature was set at approximately 32°C and then gradually reduced based on normal management practices until it reached 22°C.

### Performance and carcass parameters

Feed Intake (FI), body weight gain (BWG) and better feed conversion ratio (FCR) were measured weekly during experimental period. At end of the experiment, 8 birds (two chicks per replicate) from each treatment were randomly selected, weighed and slaughtered by cervical dislocation. The breast and thigh muscles, abdominal fat, liver, heart, gizzard and pancreas were weighed individually. The organs' weights were expressed as a percentage of live body weight.

### Morphology

On days 21 and 42 of age, two birds were randomly selected and slaughtered from each replicate. To histological morphometric analysis, formalin-fixed jejunal tissue samples were dehydrated, embedded in paraffin, sectioned (5 µM) and stained with hematoxylin and eosin. Morphometric indices, villous height and width and crypt dept, were determined on these sections by means of a computer-aided light microscopic image analyzer (Motic Images 2000 1.2, Scion Image). The small intestine length

(SIL) was also measured in centimeters by a clear plastic ruler at 21 and 42 days of age.

**Microbial sampling**

At 21 and 42 days of age, two birds were randomly selected and slaughtered from each replicate and immediately after dressing the complete intestinal tract was removed and transferred into anaerobic chamber (Hubener et al., 2002). The intestinal digesta were gently removed in sterile sampling tubes and immediately transferred on ice to microbial laboratory. Intestinal content of the two aforementioned mentioned segments were used for microbial study. Digesta were homogenized and diluted by physiological salt solution (0.9% NaCl). MRS agar (Merck, Germany) and 0.1% of Tween 80 and Violet Red Bile agar media (Merck, Germany) were used for isolation of lactic acid bacteria and *Enterobacteriaceae*, respectively. The numbers of lactic acid bacteria were calculated after incubation in an anaerobic chamber at 37°C for 48 h and *Enterobacteriaceae* were counted after aerobic incubation at 37°C for 24 h.

**Molecular assay**

Cell size and metabolic activity were estimated through measurements of mucosal protein, DNA and RNA. The relevance of the assessed biochemical indices has been described by the method of Waterlow et al. (1978). The DNA content of diploid cells remains fairly unchanged after cell formation; changes in DNA therefore reflect variations in cell population.

**Protein assay**

The protein content in the jejunum tissue was measured according to the method of Bradford (1976). In this method bovine serum albumin (BSA) was used as a standard. Samples were frozen in liquid nitrogen and ground to fine powder. Protein was extracted in 100 mg Coomassie brilliant blue + 50 ml ethanol 95% (Bradford reagent). Finally samples were measured by spectrophotometer (UV) in wave length 595 nm.

**DNA and RNA measurement**

Deoxyribonucleic acid (DNA) was extracted from crude mucosal homogenates of the jejunum, using the method described by Doyle and Doyle (1987) as follows: samples were collected from middle part of jejunum, cut and mucosa separated and transferred to micro tube. DNA extracted with cetyltrimethylammonium bromide (CTAB). Finally, DNA quantity was detected in spectrophotometer (UV) in wavelength 260 nm. For RNA measurement: RNA extraction was determined by RNX-Plus Solution® kit in mucosal homogenates of the jejunum. While for jejunum RNA extraction, samples were homogenized with liquid nitrogen. RNA was extracted with guanidinium thiocyanate (GT) and measured by spectrophotometer (UV) in wavelength 260 nm.

**Statistical analysis**

Data were subjected to analysis of variance in a completely randomized design with CRD arrangement using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 2004). When treatment means were significant (P<0.05), Duncan's multiple range test (Duncan, 1955) was used. Percentage data were transformed to arcsine percentages prior to analysis.

## RESULTS AND DISSCUSION

The effects of treatments on performance of broiler chickens are shown in Table 2. Significant higher FI was found by antibiotic, organic acids, shallot, shallot and yarrow mixture treatments as compared with the control group during the starter (0 to 21 days) period (P<0.05), but no significant reaction was shown by yarrow treatment on FI in starter period (P>0.05). Significant greater FI was observed by all treatments in comparison to control group during grower (22 to 42) and the entire (0 to 42 days) periods (P<0.05). Results of this study also indicated that BWG of treatments containing shallot and antibiotic was increased than others during starter period (P<0.05). Significantly increased BWG was obtained by supplementation of each of these additive into the diets at grower period (P<0.05). However, significantly altered BWG was shown by treatments containing shallot, shallot and yarrow mixture and antibiotic than others in the entire period. In addition, better FCR was observed for all treatments compared to control group in starter period (P<0.05). Also, FCR of treatments containing shallot, shallot and yarrow mixture and antibiotic was more suitable than others during grower and entire periods (P<0.05). The results as aforementioned are in line with those of Chowdhury et al. (2009) who reported that organic acid (citric acid, 0.5%) increased live weight compared with control group at 21 days. Also, the increased growth observed by organic acid in this study is in agreement with the results reported by Abdel-Fattah et al. (2008).They found that the addition of dietary citric, acetic or lactic acid improved live BWG of broiler chicks as compared with those fed unsupplemented diets. Similar results were found by other researchers (Moghadam et al., 2006; Nezhad et al., 2007; Talebi et al., 2010). It is well known that antibiotic improve live performance of animal and poultry. In this term, current results related to antibiotic were in contrast to the findings of Buchanan et al. (2010) and Olugbemi et al. (2004) who suggested supplementation of antibiotics to diet had no effect on performance of broiler chickens. It was however reported that organic acid and antibiotic increase metabolisable energy and protein digestibility, thus resulting in improvement in performance of poultry (Izat et al., 1990, 1991). This disagreement probably could be due to the age of birds and type of antibiotic. Present results are in disparity with Rahmatnejad et al. (2009), who reported that herbal additives (garlic and turmeric powders) had no effect on performance of broiler chickens. These diffe-rences may be related to types of herbal additive. It is noticeable that rare studies have evaluated the effect of shallot and yarrow on broiler performance until now. The effects of treatments on carcass characteristics of broiler chickens at 21d are shown in Tables 3 and 4, respectively. It was observed that no significant effect was achieved by treatments on carcass yield and pancreas percentage at day 21 (P>0.05). Breast yield of control and organic acids

**Table 2.** The effects of treatments on performance of broiler chickens.

| Treatment | FI[1] | | | BWG[2] | | | FCR[3] | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 to 2 day | 22 to 42 day | 0 to 42 day | 0 to 21day | 22 to 42 day | 0 to 42 day | 0 to 21 day | 22 to 42 day | 0 to 42 day |
| Control | 966.94[b] | 3573.68[c] | 4793.32[c] | 556.67[b] | 1476.12[b] | 2032.79[c] | 1.737[a] | 2.421[a] | 2.358[a] |
| Organic acid | 1018.37[a] | 3830.72[b] | 5163.13[a] | 607.98[b] | 1720.90[a] | 2328.88[b] | 1.675[c] | 2.226[a] | 2.217[a] |
| shallot | 1050.97[a] | 3948.63[a] | 5073.46[ab] | 663.49[a] | 1846.88[a] | 2510.37[a] | 1.584[e] | 2.138[b] | 2.021[b] |
| yarrow | 982.83[ab] | 3897.31[b] | 5198.08[a] | 575.43[b] | 1674.82[a] | 2250.25[b] | 1.708[b] | 2.327[a] | 2.310[a] |
| Shallot + Yarrow | 1048.79[a] | 4026.27[a] | 5219.26[a] | 642.25a[b] | 1824.32[a] | 2466.57[a] | 1.633[d] | 2.207[b] | 2.116[b] |
| Antibiotic* | 1047.26[a] | 4005.18[a] | 4986.32[b] | 686.28[a] | 1894.60[a] | 2580.91[a] | 1.526[f] | 2.114[b] | 1.932[c] |
| P-value | 0.0106 | 0.0064 | 0.0045 | 0.0015 | 0.0206 | 0.0027 | 0.0001 | 0.0027 | 0.0001 |
| SEM | 9.654 | 14.564 | 38.642 | 5.278 | 19.548 | 25.642 | 0.031 | 0.034 | 0.065 |

[1] FI= Feed intake; [2] BWG= body weight gain; [3] FCR = feed conversion ratio;*virginiamycin. [a,b, c, d, e, f] Means in each column with different superscripts are significantly different (p<0.05).

**Table 3.** The effects of treatments on carcass characteristic of broiler chickens at day 21 (% of live weight).

| Treatment | Carcass | Breast | Tight | AF | Liver | Heart | Gizzard | Panc |
|---|---|---|---|---|---|---|---|---|
| Control | 51.25 | 18.84[a] | 9.38[a] | 0.44[d] | 2.97[b] | 0.85[a] | 3.44[a] | 0.37 |
| Organic acid | 52.89 | 20.72[a] | 9.10[a] | 1.12[ab] | 3.63[a] | 0.83[a] | 2.56[b] | 0.42 |
| Shallot | 49.35 | 14.07[b] | 7.57[b] | 0.77[c] | 1.97[d] | 0.65[b] | 2.50[b] | 0.37 |
| Yarrow | 43.83 | 14.65[b] | 6.86[b] | 1.23[a] | 2.7[bc] | 0.59[b] | 2.60[b] | 0.41 |
| Shallot + Yarrow | 43.91 | 14.20[b] | 7.49[b] | 0.99[bc] | 2.20[cd] | 0.60[b] | 2.64[b] | 0.35 |
| Antibiotic* | 44.05 | 15.02[b] | 6.70[b] | 0.90[bc] | 2.20[cd] | 0.60[b] | 2.40[b] | 0.35 |
| SEM | 1.39 | 0.63 | 0.24 | 0.06 | 0.13 | 0.02 | 0.09 | 0.01 |
| p-values | 0.192 | 0.000 | <.000 | <.000 | <.000 | 0.005 | 0.006 | 0.445 |

[a,b,c,d] Means in same column with different superscripts are significantly different (p<0.05). LW = Live weight; AF= abdominal fat; Panc= pancreas; *virginiamycin.

treatments were significantly higher than others at day 21 (P<0.05). Tight yield and heart percentage were also followed similar pattern. Significantly lower abdominal fat was indicated by control group in comparison to others at day 21 (P<0.05). Also, lower percentage of gizzard was observed by control at day 21 (P<0.05). All parameters of carcass were not significant at day 42 (P>0.05). These results are in line with those of Chowdhury et al. (2009) who reported that organic acid (citric acid, 0.5%) had no significant effect on carcass yield at day 42. Similar results were also shown by Abdel-Fattah et al. (2008). In contrast however, current results related to abdominal fat and pancreas percentages are in disagreement with these researchers, who reported that control group had higher percentage of abdominal fat and lower pancreas percentage than birds fed the diet supplemented with organic acids. On the other hand, these results are supported by the findings of Ashayerizadeh et al. (2009), who reported that inclusion of antibiotic into broiler diets improved carcass yield, percentages of breast and tight. Furthermore, the effects of treatments on intestinal morphological indices of broiler chickens are shown in Table 5. No significant effect was observed in small intestine length (SIL) at day 21

**Table 4.** The effects of treatments on carcass characteristic of broiler chickens at day 42 (% of live weight).

| Treatment | Carcass | Breast | Tight | AF | Liver | Heart | Gizzard | Panc |
|---|---|---|---|---|---|---|---|---|
| Control | 58.53 | 21.68 | 10.09 | 1.94 | 2.36 | 0.57 | 0.41 | 0.26 |
| Organic acid | 58.89 | 21.24 | 9.28 | 1.71 | 2.70 | 0.56 | 0.40 | 0.26 |
| Shallot | 62.37 | 20.08 | 9.80 | 1.83 | 2.71 | 0.61 | 0.43 | 0.22 |
| Yarrow | 57.43 | 20.08 | 9.64 | 1.87 | 2.56 | 0.58 | 0.42 | 0.24 |
| Shallot + Yarrow | 58.62 | 20.51 | 9.94 | 2.12 | 2.68 | 0.61 | 0.40 | 0.23 |
| Antibiotic* | 57.00 | 19.20 | 9.90 | 2.10 | 2.50 | 0.62 | 0.742 | 0.22 |
| SEM | 1.00 | 0.27 | 0.10 | 0.07 | 0.05 | 0.01 | 0.00 | 0.01 |
| p-values | 0.747 | 0.091 | 0.280 | 0.554 | 0.439 | 0.654 | 0.574 | 0.230 |

AF = Abdominal fat; Panc = pancreas; *virginiamycin.

**Table 5.** The effects of treatment on intestinal morphological indices of broiler chickens.

| Treatment | 21 day | | | | 42 day | | | |
|---|---|---|---|---|---|---|---|---|
| | SIL | V.H | V.W | C.D | SIL | V.H | V.W | C.D |
| Control | 145.68 | 598.47[b] | 71.29[b] | 75.18[b] | 181.77[c] | 813.34 | 80.03[b] | 121.70[b] |
| Organic acid | 147.98 | 660.33[b] | 75.83[b] | 81.87[b] | 185.50[bc] | 871.73 | 83.02[b] | 135.23[b] |
| Shallot | 145.28 | 927.32[a] | 111.42[a] | 117.21[a] | 200.70[ab] | 1267.5 | 120.78[a] | 196.52[a] |
| Yarrow | 147.85 | 620.66[b] | 74.52[b] | 81.18[b] | 207.50[a] | 882.87 | 85.38[b] | 133.70[b] |
| Shallot + Yarrow | 145.80 | 600.50[b] | 69.35[b] | 80.69[b] | 189.25[bc] | 845.68 | 81.04[b] | 134.12[b] |
| Antibiotic* | 146.02 | 692.87[b] | 78.22[b] | 79.92[b] | 188.32[bc] | 900.61 | 83.13[b] | 138.40[b] |
| SEM | 1.87 | 35.71 | 4.38 | 4.13 | 3.05 | 48.60 | 4.29 | 7.48 |
| p-values | 0.980 | 0.0445 | 0.0345 | 0.0174 | 0.018 | 0.0515 | 0.0246 | 0.0349 |

[a,b,c]Means in same column with different superscripts are significantly different ($p<0.05$). SIL = Small intestine length (Cm); V.H = villus height (µm); V.W = villus width (µm); C.D = crypt depth (µM); *virginiamycin.

(P>0.05). Moreover, significantly longer intestine was observed by shallot and yarrow treatments compared to the control group at day 42 (P<0.05). These results are in line with the findings of Abdel-Fattah et al. (2008). Villus height and width and crypt depth of bird fed with shallot diet was significantly greater than others at day 21 (P<0.05). In addition, villus width and crypt depth followed similar pattern at day 42. More also, no reflection was obtained in villus height by treatments at day 42 (P>0.05). Present results regarding to organic acid are therefore in disagreement with Garcia et al. (2007), who reported that broiler chickens fed by formic acid had the greater villus height, width and crypt depth compared to control group. The reason of these differences may be due to type of organic acids in these experiments. Few studies have evaluated the effect of shallot and yarrow on intestinal morphology of broiler chickens in literatures.

The effects of treatments on microbial population of ileum and cecum of broiler chickens are presented in Table 6. Significant increase in lactic acid bacteria count in ileum and cecum of broiler chicken were monitored by all treatments as compared to the control at day 21 (P<0.05). Also, *Enterobacteriaceae* counts in ileum and cecum of broiler chicken fed additives were significantly lower than the control (P<0.05). All treatments ileum and significantly decreased *Enterobacteriaceae* counts in cecum of broiler chicken in comparison to the control at day 21 and 42 (P<0.05). Also, significant effect on lactic acid bacteria counts was recognized by dietary treatments at day 42 (P<0.05). In this term, higher lactic acid bacteria counts were obtained by treatments containing organic acids, antibiotic and yarrow as compared with shallot and shallot and yarrow mixture treatments, although there were no significant different between control group and others. These results are in accordance with the findings of Izat et al. (1990) and Vogt and Matthes, (1981), who reported that inclusion of organic acids and antibiotic into chicken's diet improved intestinal microbial population.

Finally, the effect of dietary treatments on protein and nucleic acids content in mucosa of broilers are illustrated in Table 7. Protein, DNA and RNA content were not affected by dietary treatments at 21 and 42 days of age (P>0.05). These results are in disagreement with the findings of Sakata et al. (1987), who suggested that organic acid causes the increase of peptide hormone secretion, thus resulting in significant increase in protein content at 42 days of age. This disagreement may be due

**Table 6.** The effect of treatments on microbial population ((log CFU/ gram digesta) of broiler chickens.

| Treatment | 21 day | | 42 day | |
|---|---|---|---|---|
| | LAB[1] | EB[2] | LAB | EB |
| Control | 7.20[c] | 7.87[a] | 10.32[ab] | 9.45[a] |
| Organic acid | 8.25[ab] | 6.75[bc] | 10.82[a] | 8.77[b] |
| Shallot | 7.72[bc] | 7.15[b] | 10.00[b] | 8.50[b] |
| Yarrow | 8.27[ab] | 7.00[bc] | 10.47[a] | 8.90[b] |
| Shallot + Yarrow | 8.05[ab] | 7.17[b] | 9.72[b] | 8.92[b] |
| Antibiotic* | 8.52[a] | 6.55[c] | 10.95[a] | 8.60[b] |
| SEM | 0.1215 | 0.1055 | 0.1262 | 0.0881 |
| p-values | 0.0075 | 0.0006 | 0.0185 | 0.0017 |

[a,b,c] Means in each column with different superscripts are significantly different ($p<0.05$). [1] Lactic acid bacteria; [2] *Enterobacteriaceae*; * virginiamycin.

**Table 7.** The effect of treatments on protein and nucleic acids content in mucosa of broiler chickens.

| Treatment | 21 day | | | 42 day | | |
|---|---|---|---|---|---|---|
| | Protein[1] (µg/mg) | DNA[2] (ng/mg) | RNA[3] (ng/µL) | Protein | DNA | RNA |
| Control | 854 | 3570 | 3520 | 1803 | 4126 | 3794 |
| Organic acid | 931 | 4202 | 3556 | 1900 | 4168 | 3954 |
| Shallot | 867 | 3948 | 3406 | 1785 | 4121 | 3742 |
| Yarrow | 872 | 4126 | 3368 | 1804 | 4339 | 3842 |
| Shallot + Yarrow | 848 | 3955 | 3404 | 1766 | 4102 | 3682 |
| Antibiotic* | 909 | 4105 | 3544 | 1806 | 4349 | 3682 |
| SEM | 17.69 | 82.00 | 29.59 | 17.69 | 64.43 | 42.37 |
| p-values | 0.7440 | 0.2802 | 0.2653 | 0.4776 | 0.8073 | 0.4322 |

*Virginiamycin

to type and antibiotic levels in diet.

## Conclusion

The results of this study revealed that supplementation of antibiotic, organic acids and herbal additives improved performance and morphological indices of broiler chickens. In addition, these results showed that dietary treatments had no significant effect on protein and nucleic acids content in intestinal mucosa of broilers. Finally, organic acids and herbal additive such as shallot and yarrow might be useful additive instead of antibiotic growth promoters such as virginiamycin, considering performance, intestinal morphology and ileal microbial population of broilers.

### REFERENCES

Abdel-Fattah SA, EI-Sanhoury MH, EI-Mednay NM, Abdul-Azeem F (2008). Thyroid activity of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7: 215-222.

Ashayerizadeh A, Dabiri N, Ashayerizadeh O, Mirzadeh KH, Roshanfekr H, Mamooee M (2009). Effect of dietary antibiotic, probioticand prebiotic as growth promoters, on growth performance, carcasscharacteristics and hematological indices of Broiler Chickens. Pak. J. Biol. Sci. 12: 52-57.

Barel S, Segal R, Yashphe J (1991). The antimicrobial activity of the essential oil from *Achillea fragrantissima*. J. Ethnopharmacol. 33: 187-191.

Blumenthal M, Busse M, Goldberg A (1998). The complete german commission emonographs: therapeutic guide to herbal medicines. American Botanical Council. USA. pp. 55-56.

Botsoglu NA, Fletouris DJ (2001). Drug resistant in foods pharmacology, food safty and analysis. Marcel Dekker Inc. New York. pp. 541-548.

Bradford M (1977). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal. Biochem. 72: 248-254.

Buchanan NP, Lilly KGS, Moritz JS (2010). The effects of diet formulation, manufacturing technique, and antibiotic inclusion on broiler performance and intestinal morphology. J. Appl. Poult. Res. 19: 121-131.

Chevallier A (1996). The encyclopedia of medicinal plants. Dorling Kindersley Publishers. London.

Denil M, Okan F, Celik K (2003). Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pak. J. Nutr. 2: 89-91.

Dibner JJ, Buttin P (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11: 453-463.

Doyle JJ, Doyle JJ (1987). Rapid DNA isolation procedure for small quantities of fresh leaf tissue. Pithchemecal Bull. 9:11-15.

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

Garcia V, Catala-Gregori P, Hernandez F, Megias MD, Madrid J (2007). Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. J. Appl. Poult. Res. 16: 555-562.

Harris JC, Cottrell SL, Plummer S, Lloyd D (2001). Antimicrobial properties of Allium sativum (garlic). Appl. Microbiol. Biotechnol. 57: 282-286.

Hubener K, Vahjen W, Simon O (2002). Bacterial responses to different dietary cereal types and xylanase supplementation in the intestine of broiler chicken. Arch. Anim. Nutr. 56: 167-187.

Izat AL, Adams HM, Cable MC, Colberg M, Reiber MA, Slinder JT, Walderoup PW (1991). Effect of formic acid or calcium formate in feed on performance and microbiological characteristic of broilers. Poult. Sci. 61: 2224-2231.

Izat AL, Tidwell NM, Thomas RA, Reiber MA,Adams MH, Colberg M, Waldroup PW (1990). Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. Poult Sci. 69: 818-826.

McCartney E (2002). The natural empire strcks book. Poult. Int. 41: 36-42.

Moghadam AN, Pourreza J, Samie AH (2006). Effect of different levels of citric acid on calcium and phosphorus efficiencies in broiler chicks. Pak. J. Biol. Sci. 9: 1250-1256.

National Research Council (1994). Nutrient reuirements of poultry. 9th Rev. Edition. Natl. Acad. Sci. Washington. DC.

Nezhad YE, Shivazad M, Nazeeradl M, Babak MMS (2007). Influence of citric acid and microbial phytase on performance and phytate utilization in broiler chicks fed a corn-soybean meal diet. J. Fac. Vet. Med. Univ. Tehran. 61: 407-413.

Olugbemi TS, Ubosi CO, Akpa GN, Esuga WH (2004). Response of broilers to antibiotic and antistress dietary inclusion. Pak. J. Nutr. 3(4): 262-263.

Chowdhury R, Islam KMS, Khan MJ, Karim MR, Haque MN, Khatun M, Pesti GM (2009). Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers. Poult. Sci. 88: 1616–1622.

Rahmatnejad E, Roshanfekr H, Ashayerizadeh O, Mamooee M, Ashayerizadeh A (2009). Evaluation the effect of several non-antibiotic additives on growth performance of broiler chickens. J. Anim. Vet. Adv. 8(9): 1757-1760.

Sakata T (1987). Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Br. J. Nutr. 58: 95-103.

SAS Institute (2004). SAS® User's Guide Statistics. Version 9.1. SAS Institute, Inc., Cary, NC.

Shokouhi SJF, Tajik H, Tehrani A (2007). Experimental evaluation of repair process of burn wound treated with aqueous extract of *Achillea millefolium* on animal model: Clinical and histopathological study. J. Anim. Vet. Adv. 6: 1357-1361.

Talebi E, Zarei A, Abolfathi ME (2010). Influence of three different organic acid on broiler performance. Asian. J. Poult. Sci. 4(1): 7-11.

Taran M, Rezaeian M, Izaddoost M (2006). *In vitro* antitrichomonas activity of *Allium hirtifolium* (Persian Shallot) in comparison with metronidazole. Iranian J. Publ. Health. 35: 92-94.

Vogt H, Matthes S (1981). Effect of organic acids in ration on the performances of broilers and laying hens. Arch. Gefluelkd. 45: 221-232.

Waterlow JC, Garlick PJ, Millward DJ (1978). Protein turnover and growth, in protein turnover in Mammalian tissues and in whole body. North-Holland, Amsterdam, pp. 529-594.

William P, Losa R (2001). The use of essential oils and their compounds in poultry nutrition. World Poult. 17: 14-15.