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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 measure-ments 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.

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