IJAS Iranian Journal of Applied Animal Science

Growth Performance and Biochemical Parameters of Broiler Chickens on Diets Consist of Chicory (*Cichorium intybus*) and Nettle (*Urtica dioica*) with or without Multi-Enzyme

Research Article

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Received on: 20 Jan 2012
Revised on: 17 Mar 2012
Accepted on: 2 Apr 2012
Online Published on: Mar 2013

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Online version is available on: www.ijas.ir

ABSTRACT

A total of 288 broiler chickens (1 d old) were used in a 42-d growth trial to study the effects of chicory (*Cichorium intybus*) and nettle (*Urtica dioica*) with or without multi-enzyme on growth performance, carcass traits, and biochemical parameters. The corn-soybean based control diet and 8 experimental diets were fed to each of 3 pens of chickens (12 chickens/pen). Treatments were as follows: 1. Basal diet (B), no additives, 2. B + 0.05% enzyme (E), 3. B + 1% chicory (C), 4. B + 1% nettle (N); 5. B + 1% N + E (NE), 6. B + 1% C + E (CE), 7. B + 0.5% N + 0.5% C (NC), 8. B + 0.5% N + 0.5% C + E (NCE). A higher body weight gain were found at 42 d of age in chickens fed the E, CE, NE, and NCE in diet (P<0.05), whereas BW did not differ between the control and C, N, and NC diets. A lower food conversion ratio was observed at 42 d of age in birds receiving the NE, and CE. No diet effects on carcass yield, and relative weights of gizzard, and ceca were detected. In contrast, a higher breast yield was found for all treatments except to N group (P<0.05). The intestinal weight decreased with E, NE, CE, NC, and NCE (P<0.05). Serum cholesterol decreased in birds fed C, and NC (P<0.05) as compared to N. Serum total protein and triglyceride content were similar among all treatments. Serum cholesterol concentration indicated the C and NC treatment had a lower concentration than N (P<0.05). Broiler performance and carcass traits for birds given dietary NE and CE supplement were similar to other treatment containing herb or enzyme or blend of them, and these supplements appear suitable for dietary inclusion. Careful choices are necessary when selecting dietary herb supplements for broilers, but beneficial effects can be observed.

KEY WORDS broiler chicken, chicory, enzyme, nettle, performance.

INTRODUCTION

In response to concerns about the transfer antimicrobial-resistant bacteria to animal production and resulting in to human food, many countries have started to control the general use of antibiotics. The search of the substitutes for antibiotic growth promoters (AGP) that are presently banned in European Union, turned the attention to many other alternative additives, including herbs. It is therefore desirable to find feed components with less risk than in-feed antibiotics, in order to reduce mortality of animals and improve the quality of animal products in feed. Phytogenic food additives are purported to promote natural digestion while improving performance along with other various modes of action such as decreasing bacterial colony counts and fermentation products (ammonia and biogenic amines), reducing activity of the gut associated lymphatic system, increasing pre-cecal nutrient digestion, and possessing anti-oxidative properties. Limited research exists surrounding the growth promoting effects of phytogenic food additives in poultry. However, Buchanan *et al.* (2008) reported that broilers fed diets containing plant extract blends (microencapsulated essential oils, bitter and pungent substances) had lower food conversion ratios, improved live weight gain, and higher breast yield (Buchanan *et al.* 2008). Windisch *et al.* (2008) completed a comprehensive review regarding phytogenic food additives and the proposed modes of actions of these products. The general conclusion encompassed in this review supports the idea that phytogenic food additives may add to the set of non antibiotic growth promoter thereby increasing animal performance naturally. It is observed that feeding chicory as a dietary fiber and inulin source has pronounced systemic effects on monogastric animals. The inclusion of fiber in the diet has either positive or negative nutritive effects on the gut health of mono gastric animals. Several studies have been carried out to describe the 'prebiotic effect' of chicory inulin type fructans and oligofructose (Castellini *et al.* 2007; Chen *et al.* 2005; Van Loo, 2007). Some herbs have been also used for a long time as feeds e.g. nettle or, causing satisfying productivity (El Deek *et al.* 2003; Mandal *et al.* 2000). Haydari *et al.* (2010) evaluated the effects of nettle, menta pulagum and zizaphora medicinal plants in broiler chickens, and confirmed the positive effects of these medicinal plants on performance, carcass traits and blood biochemical parameters of broilers.

Nettle is reported to have medical (Viegi *et al.* 2003), antioxidative (Toldy *et al.* 2005) and growth-stimulating properties.

The benefits obtained with respect to herbs as therapeutics and feeds raised the hope of applying them as food additives, instead of antibiotics. Herbs fed as the food additives are believed to improve metabolism rate and the health status in animals. It seems that some herbs can support the activity of digestive enzymes, resulting in improved feed consumption and conversion. It is also commonly believed that they are capable positively affect carcass characteristics and poultry meat traits (Dickens *et al.* 2000; El Gendi *et al.* 1994; Kinal *et al.* 1998; Pietrzak *et al.* 2005). However some researchers reported no positive influence of herbs (Halle *et al.* 2004).

On the other hand, various plant extracts, especially essential oils, have been investigated on the basis of their demonstrated *in vitro* antimicrobial activity (Deans and Ritchei, 1987; Reddy *et al.* 1991; Cowan, 1999; Hammer *et al.* 1999).

Furthermore, except for the benefits mentioned before, there was evidence that dietary fiber increased endogenous losses in intestine. As a result, the energy and nutrient digestibility were decreased in both ileal digestibility and apparently had influence on health (Souffrant, 2001; Choct and Annison, 1992).

The fiber component was also described as an "anti-nutritive" diet source due to its negative effects, especially for chickens (Eggum, 1995).

It is known that the detrimental effect is mainly related to the soluble nonstarch polysaccharide (NSP) components in dietary fiber fraction and increased digesta viscosity in intestine (Bedford and Classen, 1992). As it indicates from the bibliography, the results of experiments dealing with herbs are not univocal, due to the alternation in chemical composition of herbs (especially with respect to the bioactive constituents). Therefore, the objective of this study was to assess the use of nettle, chicory with and without an enzyme in commercial broiler diet formulation. The criteria studied included performance, carcass quality and some biochemical parameters of birds.

MATERIALS AND METHODS

An experiment was conducted with female Ross × Ross 308 broilers obtained from a commercial hatchery. All methods used in these experiments regarding animal care were approved by the Maragheh University Agricultural Center Animal Care and Use Committee. Birds were randomly assigned to 1 of 8 dietary treatments (3 replicates; 12 birds/pen) and were raised on floor pens (10 birds/m^2). Trial lasted 42 days from 1 to 42 d post hatching. The experimental diets were as follows: 1. Basal diet, no additives (Control), 2. Basal diet + enzyme (E, 0.05% of diet), 3. Basal diet + 1% chicory (C), 4. Basal diet + 1% nettle (N); 5. Basal diet + 1% nettle + E (NE), 6. Basal diet + 1% chicory + E (CE), 7. Basal diet + 0.5% nettle + 0.5% chicory (NC), 8. Basal diet + 0.5% N + 0.5% C + E (NCE). All diets were in C-SBM based, mash form, and were formulated to be isoenergetic and isonitrogenous, and to meet or exceed nutrient requirements for macro- and micronutrients (Table 1). Fresh chicory and nettle were purchased from Shafanosh Co. (Maragheh, Iran), sun-shade dried and then ground to obtain their powder. The Kemzyme® (Zobin-Aria Co; Tehran, Iran) was used as multi-enzyme. The experimental diets and drinking water were provided *ad libitum*. Throughout the study, birds were housed in an environmentally controlled room under a 23 L: 1D lighting cycle and following a standard temperature regimen, which gradually decreased from 32 to 23 ˚C by 3 ˚C weekly. Birds were group weighed by pen, and feed intake was determined at weekly intervals.

At the end of the experiment (at d 42), 6 birds whose body weights were close to the group average were selected from each of the replicate groups of treatments. These birds were slaughtered by CO_2 asphyxiation to determine some measurements of carcass yield, breast, thigh, selected internal organs, abdominal fat pad, liver, heart, gizzard, duodenum, intestinal, cecum. The hot carcass yields were calculated as percentages of the pre slaughter live body weight of broiler chickens. After 12 h of fasting, blood samples were collected in non-heparinised tubes at 42 days of age from 16 birds in each treatment by puncturing the brachial vein and the blood was centrifuged at 2000 × g for 15 min to obtain serum (SIGMA4-15 Lab Centrifuge, Germany).

Table 1 Ingredients and chemical composition of the experimental diets[1] (as fed)

Ingredients	0 - 21 days		21 - 42 days	
	T1	T3	T1	T3
Corn	58.51	57.12	65.61	64.27
Soybean meal	31.72	32.77	29.43	29.43
Fish meal	3	3	0	0
Vegetable oil	2.53	2.89	1.60	1.96
Nettle or chicory	0	1	0	1
Oyster shell	1.30	1.27	1.28	1.28
Di calcium phosphate	1.06	1.07	1.25	1.24
Iodized salt	0.23	0.23	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25
Mineral premix[3]	0.25	0.25	0.25	0.25
DL-methionine	0.15	0.15	0.05	0.05
Calculated composition[4]				
Metabolizable energy[5]	12.55	12.55	12.55	12.55
Crude protein	21.56	21.56	18.75	18.75
Calcium	0.94	0.94	0.85	0.85
Available phosphorus	0.42	0.42	0.38	0.38
Sodium	0.14	0.14	0.14	0.14
Linoleic acid	1.42	1.40	1.55	1.55
Crude fiber	3.71	3.88	3.71	3.88
Lysine	1.25	1.25	1.02	1.02
Met + Cys	0.87	0.87	0.67	0.67
Determined Analysis[4,6]				
Crude protein	19.45	19.82	17.45	17.5
Crude fiber	3.53	3.75	3.5	3.7
Calcium	0.85	0.87	0.82	0.84
Total phosphorus	0.5	0.51	0.51	0.52

[1] T1: the basal diet; T2: T1 supplemented with enzyme (E); T3: the basal diet supplemented with chicory (C); T4: T3 supplemented with nettle (N); T5: T3 supplemented with N + E; T6: T3 supplemented with C + E; T7: T3 supplemented with C + N; T8: T3 supplemented with C + N + E.

[2] Vitamin premix (mg/kg diet): vitamin A: 12000 IU; vitamin D: 1500 IU; vitamin E: 30 mg; vitamin K_3: 5 mg; vitamin B_1: 3 mg; vitamin B_2: 6 mg; vitamin B_6: 5 mg; vitamin B_{12}: 0.03 mg; Nicotine amid: 40 mg; Calcium-D-pantothenate: 10 mg; Folic acid: 0.75 mg; Biotin: 0.075 mg; Choline chloride: 375 mg and Antioxidant: 10 mg.

[3] Mineral premix (mg/kg diet): Mn: 80; Fe: 80; Zn: 60; Cu: 8; I: 0.5; Co: 0.2 and Se: 0.15.

[4] As a percentage except to ME.

[5] Mj/kg.

[6] n= 2.

Individual serum samples were analyzed for total protein, cholesterol, triglyceride, and glucose using the kit package (Pars Azmoon Co; Tehran, Iran). Data were analyzed by ANOVA procedures (Steel and Torrie, 1980) appropriate for completely randomized designs using the GLM procedure of SAS (1998). The pen of broilers served as the experimental unit. Treatment's means were compared using Tukey at the level of P<0.05 significant.

RESULTS AND DISCUSSION

In general, no difference in weight gain (g), and food conversion ratio (FCR) was observed in female broilers fed with different diets at 21 d of age (Table 2). During 21 to 42 d of age, birds fed the diets containing one of treatments had greater weight gains (P<0.05) than birds fed control diet. However, no effect of supplemented diets was detected for feed intake (g) at whole periods. For the overall experimental period (0-42 d), BW increased in treatment CE, NE, NCE, and E, when compared with the control (Table 2). The NE, and CE supplementation improved FCR of birds at 42 d of age in comparison with control (P<0.05), however, there found no significant differences in FCR between the different concentrations of C or N, and E supplementation (Table 2). There is evidence to suggest that herbs, spices and various plant extracts have appetite- and digestion-stimulating properties and antimicrobial effects (Jain *et al.* 2008). Indeed, herbs can stimulate the production of secretions in the small intestinal mucosa, pancreas and liver, which leads to help digestion. Other proposed mechanisms for herbs include increased availability at the intestinal brush border (Khajuria *et al.* 2002) or affected glucose metabolism (Roman-Ramos *et al.* 1995). The positive effect of NE, CE, and CNE supplementation on BW and FCR (P<0.05) could be attributed to the effect of bioactive compounds (aromatic compounds and essential oils) of herbs (Kamel *et al.* 2001), and its beneficial effect on the utilization of nutrients, and partially to the effect of enzyme in ameliorate of anti nutritional factors. Similarly, Lee *et al.* (2003) and Ocak *et al.* (2008) reported that using 2% thyme supplement could significantly improve the growth of broilers. In the other preliminary study it has been demonstrated that the inclusion of ground hops into broiler diets at the rate of 0.45 kg per ton significantly improved growth rate and food utilization in the absence of growth promoting antibiotics (Cornellison *et al.* 2006). Blends of essential oils from herbs have improved broiler performance (Suk *et al.* 2003; Hernandez *et al.* 2004). In other studies, only very limited information is available concerning the composition of the blend. In contrast, Botsoglou *et al.* (2004) showed that oregano oil exerted no growth-promoting effect when administered at 50 or 100 mg/kg of feed. Likewise, No significant differences on body weight gain of broilers were observed when thyme powder (Sarıca *et al.* 2005) antibiotic plus enzyme containing β-glucanase and xylanase (Vukic-Vranjes and Wenk, 1995) and a blend of extracts of sage, thyme and rosemary (Hernandez *et al.* 2004) were added to a diet. Improved feed utilization (FCR) was observed at 42 d of age in chickens fed diet CE, and NE compared with those fed diet control, whereas the FCR was comparable between the C, N, CN, E and CNE diets.

Table 2 The effects of inclusion an enzyme, chicory and nettle in broiler diets on performance

Treatments	Age (day)	T1	T2	T3	T4	T5	T6	T7	T8	SEM
Weight gain (g)	0-21	12.09	11.34	11.58	12.35	13.04	11.80	12.55	12.67	7.55
	21-42	14.96^{b}	21.02^{a}	19.67^{a}	20.99^{a}	22.72^{a}	22.19^{a}	20.60^{a}	21.83^{a}	1.72
	0-42	38.05^{b}	44.83^{a}	42.29^{ab}	44.47^{ab}	46.41^{a}	45.25^{a}	44.41^{ab}	45.70^{a}	3.3
Feed intake (g)	0-21	20.53	22.75	22.42	22.82	22.70	22.55	22.58	22.51	1.75
	21-42	40.12	44.92	42.99	42.51	41.35	40.66	41.63	43.63	3.85
	0-42	76.62	85.40	81.87	82.20	80.84	78.45	80.20	83.24	8.65
Feed conversion ratio	0-21	1.69	2.11	1.95	1. 95	1.74	1.84	1.74	1.82	0.16
	21-42	2.68^{a}	2.13^{b}	2.18^{b}	2.04^{b}	1.82^{c}	1.83^{c}	2.15^{b}	2^{ab}	0.06
	0-42	2.01^{a}	1.91^{ab}	1.94^{ab}	1.87^{ab}	1.74^{b}	1.73^{b}	1.81^{ab}	1.81^{ab}	0.09
Mortality	0-42	0.78	0.86	0.86	0.78	0.95	0.92	0.81	0.86	0.06

* T1: the basal diet; T2: the basal diet supplemented with enzyme (E); T3: the basal diet supplemented with chicory (C); T4: the basal diet supplemented with nettle (N); T5: the basal diet supplemented with N + E; T6: the basal diet supplemented with C + E; T7: the basal diet supplemented with C + N; T8: the basal diet supplemented with C + N + E.

** The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of mean.

In our study, a significant effect of both enzyme and herbs on performance was found only in the second 3 weeks suggesting that beneficial effects might have been more pronounced if the supplementation period had been prolonged. Our results are in agreement with Alcicek *et al.* (2004) and Çabuk *et al.* (2006) who observed significant improvements on FCR in 42-d-old broilers fed a diet supplemented with the herbal essential oil.

There are limited reports on the performance of broilers fed corn-based diets supplemented with nettle, chicory, with or without an enzyme. Feed intake was not affected by any of treatments that are consistent with other studies (Hernandez *et al*. 2004; Basmacioğlu *et al*. 2004). Performance data from the current study also revealed that the addition of an enzyme complex to the corn-based diets with herbs did not lead to significant improvement in performance of the broilers.

This response in bird performance to dietary enzyme addition agrees with the findings of other researchers (Choct *et al*. 1999; McCracken and Bedford, 2001), who reported either no response or a negative response to the effects of xylanases on broiler performance. Mortality was not affected (P>0.05) by the treatments. The lack of response to herbal food additives on broiler livability in different studies, was attributed to clean, hygienic and unstressed housing conditions (Guo *et al*. 2004; Sarica *et al*. 2005).

Dietary treatment had no effect on the carcass yield, and the relative weight of gizzard, heart, liver and cecum (Table 3). The birds fed on diets with enzyme supplementation had the largest carcass weight (%), although there was no difference between whole dietary supplements. The breast weight (%) was increased for the birds given all dietary supplements, except to those receiving the N group, as compared with the control (P<0.05). Somaieh *et al.* (2001) showed that the use of different levels of nettle in starter and grower feeds had significant effects on carcass traits of broilers (P<0.05).

As in our results, Ocak *et al*. (2008) found that broilers given dietary thyme powder at 2% of diet showed no differences in the relative weight of the organs. A similar observation was reported by Ceylan *et al*. (1998), who concluded that carcass yield was not affected by either enzyme or enzyme plus antibiotic treatment compared to control diet. In addition to, the birds given C in their diet had a greater relative thigh weight (%) than those receiving CE; whereas, the difference between all groups with the control diet were not significant. The average abdominal fat of chicks fed N and N plus E was 2.49, and 2.68%, respectively, which were lower than C, CE, CN, CN and CNE. In general, body fat accumulation may be considered as the net result of the balance among dietary absorbed fat, fat synthesis (lipogenesis) and fat catabolism via β-oxidation (lipolysis).

Therefore, if the amount of absorbed fat is the same, lower body fat deposition might be attributed to increased fat catabolism or diminished fatty acid synthesis or to both processes. This result is in contrast with the results of Sizemore and Siegel (1993), who did not find any effect of dietary fat concentration when the energy to protein ratio remained constant. This effect was more pronounced in birds fed nettle at level of 1%. Only a tendency towards a slightly higher carcass meat content and breast meat correspond with indices of low fat abdominal.

There was significant reduction in intestinal, and duodenum weight (Table 3) in birds fed E, CE, CN, and CNE supplementation compared with the controls (P<0.05). Henry *et al*. (1986) showed that intestinal tract weight decreased with 12 mg antibiotic virginiamycin as a result of thinning of the intestinal wall. Likewise, it has been suggested that the presence of bacteria may induce a chronic inflammation, resulting in a thickening of the intestinal wall, which, in turn, impairs intestinal absorption and decreases the amount of nutrients available for the host (Visek, 1978).

Table 3 The effects of inclusion an enzyme, chicory and nettle in broiler diets on carcass traits, and relative organ weights (organ weight/100 g BW) in broiler chickens at 42 days of age

Carcass traits	Treatments								
	T1	T2	T3	T4	T5	T6	T7	T8	SEM
Carcass (%)	70.15	72.20	72.16	71.67	71.67	71.70	71.76	72.71	0.88
Breast (%)	29.88[b]	32.71[a]	33[a]	32.50[ab]	33.44[a]	33.54[a]	35.19[a]	34.06[a]	0.87
Thigh (%)	26.79[ab]	26.65[ab]	27.28[a]	25.94[ab]	26.27[ab]	25.24[b]	25.66[ab]	25.82[ab]	0.52
Abdominal fat (%)	3.07[ab]	3.97[ab]	3.07[ab]	2.49[b]	2.68[b]	3.95[a]	3.57[a]	3.52[a]	0.33
Liver weight	3.28	3.21	3.02	3.1	2.93	3.06	2.97	3.07	0.18
Gizzard weight	3.68	3.72	4.03	3.76	4.29	4.27	3.69	3.70	0.28
Heart weight	0.87	0.76	0.89	0.80	0.87	0.89	0.84	0.89	0.06
intestinal weight	8.70[a]	6.77[bc]	7.43[abc]	7.68[ab]	6.94[bc]	6.98[bc]	6.63[bc]	6.03[c]	0.47
Duodenum weight	0.43[a]	0.28[b]	0.31[b]	0.36[ab]	0.33[b]	0.35[ab]	0.30[b]	0.32[b]	0.03
Ceca weight	1.54	1.18	1.48	1.4	1.23	1.26	1.28	1.29	0.13

* The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Other studies (Gill, 1999; Langhout, 2000; Wenk, 2000) have been shown that herbs and plant extracts stimulate the growth of beneficial bacteria and minimize pathogenic bacterial activity in the gastrointestinal tract of poultry. Therefore, the results in our study might be attributable to the effects of bioactive compounds of herbs, such as aromatic compounds and essential oils. These results indicate that herbs could have antimicrobial effects and might explain the improved FCR in herbs treatments. On the other hand, the results obtained in our study is in agreement with Viveros *et al.* (1994), who found that the addition of enzyme β-glucanase decreased the relative weight of intestinal in barley-based diets (P<0.05). There was no significant difference in triglyceride and total protein levels between the treatment groups. The reduction effect of chicory on cholesterol was greater than other treatments (Table 4).

Table 4 The effects of inclusion an enzyme, chicory and nettle in broiler diets on the blood biochemical parameters

Treatment	Biochemical parameters*			
	Glucose	Protein	Cholesterol	Triglyceride
T1	194.28[a]	4.17	130.60[ab]	51.64
T2	180[ab]	3.95	127.05[ab]	37.37
T3	179.50[ab]	3.45	105.29[b]	37.83
T4	188.68[ab]	3.58	137.18[a]	34.79
T5	171.32[ab]	3.59	117.86[ab]	41.39
T6	161.43[b]	3.53	128.01[ab]	46.30
T7	184.15[ab]	3.29	111.61[b]	49.07
T8	192.97[a]	3.62	127.47[ab]	40.80
SEM	8.7	0.37	9.07	6.08

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (mg/100 mL).

This result was supported by Elson (1995), who found that these isoprenoids suppress cholesterol synthesis by inhibiting the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate controlling enzyme of the cholesterol synthetic pathway. Moreover, Fremont *et al.* (2000) reported that the cholesterol levels in blood serum and meat were probably lowered by phenolic compounds. Meanwhile, the addition of chicory extract significantly decreased cholesterol absorption by 30% (P<0.05) in the jejunum and by 41% (P<0.05) in the ileum, as compared with control (Kim, 2000). Indeed, there were significant differences in the level of glucose, so that birds receiving C plus E showed highest reduction (P<0.05) in glucose level (Table 4), however, the difference between all other dietary supplementation was not significant (P>0.05). The reasons for such effects of herbs are not understood at the present. In general, there is a dearth of information regarding the effect of feeding chicory on the biochemical parameters of broiler chickens. Therefore, it seems that these two herbs (nettle and chicory) with enzyme could be recommended to improvement performance without any harmful effects on studied traits.

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