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Evaluation of different medicinal plants blends in diets for broiler chickens

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The effects of five blends of medicinal plants on performance, carcass characteristics, humoral immunity and serum lipids of broiler chickens were studied in this experiment. A total of 304 day- old male Ross-308 broiler chicks were allocated into six dietary treatments including basal diet with no supplement as control group (C), basal diet plus 10 g/kg of herbal blends including; garlic, cinnamon, thyme, rosemary and anise (B), thyme, caraway, carum copticum (G), alfalfa, senna, corn flower and absinthe (D) alfalfa, liquorice root, great burdock, cinnamon (F), polygermander, water cress, absinthe and echinacea purpura (E). Live body weight (LBW), average daily gain (ADG), daily feed intake (DFI), feed conversion ratio (FCR), carcass characteristics, concentration of some serum metabolites, immunological properties such as antibody titer against Newcastle disease virus as well as relative weights of bursa gland and spleen were studied in the experimental birds. Addition of blend D to the diet resulted in insignificant improvement of LBW whereas blend E decreased the birds LBW when compared with control group ($p < 0.05$) at 21 and 42 days of age. Significant depression of ADG in 1-21 and 1-42 and higher FCR in 1-42 rearing periods were also recorded in the blend E treated chickens ($p < 0.05$). The birds DFI were not affected by the experimental diets. Higher cholesterol contents of serum in B, F and G groups at day 33 and lower TG and VLDL contents at day 21 of age were noticeable changes in to the measured serum metabolites ($p < 0.05$). The addition of 10 g/kg blend F to the broiler diet resulted in the most consistent improvement in antibody titer against Newcastle disease virus ($p < 0.05$) among the groups. Lower carcass yield was documented in the administration of blend E in broiler diet than control and D treated birds ($p < 0.05$). The supplemented medicinal plants used in this study did not create significant enhancement in broiler bird's performance; however, some improvements were occurred in immunological properties and serum related parameters. In conclusion, blend D that contained alfalfa, senna, corn flower and absinthe may be a proper candidate to fulfill the demand of poultry industry in search for safe and efficient growth enhancers.

Key words: Medicinal plants, growth performance, immune system, broiler chicks.

INTRODUCTION

During the past 50 years, the growth rate of broiler chickens has been improved greatly. Feeding antibiotics as growth promoters had a substantial role in poultry industry. Currently, the global paradigm is shifting from an emphasis on productive efficiency to one of the public securities issues. The World Health Organization (WHO) has recently identified antibiotic resistance as a major problem for public health on a global scale. For this reason, an overflow of studies is triggered to introduce suitable alternatives for antibiotics. Medicinal plants and their products including plant extracts or essential oils are introduced as candidates for use in broiler diets in which

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Table 1. Composition of experimental chicken diets and calculated major components (% as fed).

Ingredients (%)	1-21 days	22-42 days
Corn grain	54.17	63.49
Soybean meal (44)	39.84	30.72
Soybean oil	2.12	1.84
$CaCo_3$	1.18	1.07
Dicalcium phosphate	1.56	1.73
Common salt	0.34	0.33
Vitamin premix[1]	0.25	0.25
Mineral premix [2]	0.25	0.25
DL- Methionine	0.20	0.27
L- Lysine HCL	0.10	0.06
Nutrients composition		
Metabolizable Energy (Mcal/ Kg)	2900	3.05
Crude protein	22.50	20.70
Crude Fiber	4.10	2.59
Calcium	0.92	0.90
Available phosphorous	0.45	0.40
Lysine	1.38	1.12
Methionine + Cystine	0.92	0.92
DCAD ($Na^+ + K^+ - Cl^-$) (meq/Kg)	241.85	201.96

*Each kilogram of vitamin supplement contains: Vitamin A, 3600000 IU, vitamin D_3, 800000 IU, vitamin E, 7200 IU, vitamin K3, 800 mg, vitamin B_1, 720 mg, vitamin B_2, 2640 mg, vitamin B_3, 4000 mg; vitamin B_5, 12000 mg, vitamin B6, 1200 mg, vitamin B_9, 400 mg, vitamin B12 , 6 mg, biotin, 40 mg, choline chloride, 100000 mg, antioxidant, 40000 mg, ** Each kilogram of mineral supplement contains: Mn, 40000 mg, Zn, 33880 mg, Fe, 20000 mg, Cu, 4000 mg, I, 400 mg, Se, 80 mg, choline chloride, 100000 mg.

their beneficial effects as phytogenic feed additives have been proven (Bölükbaşi and Erhan, 2007; Soltan et al., 2008 and Dalkiliç et al., 2009). Such compounds influence poultry productivity and health mainly by stabilization of normal gut microflora, prevention of pathogens colonization (Tekeli et al. 2006) and digestive enzymes production and activities improvement(Lee et al, 2004).

They also exert certain immunological consequences in bird's body (Kong et al., 2006). Lots of studies on phytogenic compounds of plants essential oils have been performed while there are limited evidences about the effect of herbal solid forms on live birds health and performance. Easy and practical application, availability and less cost are known as advantages of the whole herbs application in compare to extracted or essential oil forms. In the other hand, a synergistic effect of phytogenic compounds have been reported in studies with essential oils (Mitsch et al., 2004), and a combination of herbal powders might tends to be more effective than a single herb administration.

Therefore, this study was carried out to evaluate the possible growth enhancer effects of five blends of medicinal plants, according to traditional medicine literatures, in male broiler chicks. The blends were selected for their potential benefits to birds' appetite, digestion, immunity and antibacterial activities.

MATERIALS AND METHODS

Birds

A total of 304 Ross-308 one day old male broilers were provided by a local broiler breeder company, and randomly allocated into six dietary treatments. Each treatment was replicated four times with 16 birds per each. The birds were given 23 L: 1 D lighting program during each 24 h period throughout the 42 days of trial.

Diets

Starter and grower diets were offered from 1 to 21 and 22 to 42 days of ages, respectively. Feed and water were provided *ad libitum* throughout the experiment. All diets were presented to the birds as mash. The composition and nutrients content of the basal diets is shown in Table 1. The diets were formulated to meet or exceed the National Research Council (NRC,1994) requirements. A basal diet with no additives considered as control (C), and five experimental treatments were formulated by supplementation of; 10 g/kg of five separate herbal blends to the diets. The supplemented herbal blends were prepared with equal ratio as follow: garlic, thyme leaf, cinnamon, rosemary leaves, anise (B); alfalfa leaves, corn flower, senna leaves, absinthe (D); echinacea purpurea, water cress, absinthe, polygermander (E); alfalfa leaves meal, cinnamon, burdock root, licorice root (F); and thyme, caraway, carum copticum (G).

Table 2. Effect of medicinal plant blends on performance of broiler chickens at 21 and 42 days of age.

Parameters	Medicinal plant blends[1]						
	C	B	D	E	F	G	SEM
Live body weight (g)							
21 day	763.06 ^{ab}	773.54 ^{ab}	818.93 ^{a}	660.32 ^{c}	719.33 ^{bc}	741.07 ^{ab}	10.961
42 day	2469.98 ^{ab}	2500.54 ^{a}	2574.59 ^{a}	2253.06 ^{c}	2299.58 ^{bc}	2402.76 ^{abc}	32.513
Daily weight gain (g)							
1-21 day	33.66 ^{ab}	34.64 ^{ab}	36.33 ^{a}	29.58 ^{c}	32.15 ^{bc}	33.44 ^{ab}	0.605
22-42 day	82.24	82.73	82.86	76.53	76.10	79.73	0.992
1-42 day	55.84 ^{ab}	56.87 ^{a}	57.56 ^{a}	51.36 ^{c}	52.38 ^{bc}	54.63 ^{abc}	0.636
Daily feed intake (g)							
1-21 day	52.72 ^{ab}	54.11 ^{a}	54.78 ^{a}	50.51 ^{b}	52.32 ^{ab}	52.19 ^{ab}	0.475
22-42 day	164.87 ^{ab}	170.98 ^{a}	170.04 ^{ab}	162.73 ^{ab}	159.96 ^{b}	160.71 ^{ab}	1.446
1-42 day	103.94 ^{ab}	108.13 ^{a}	107.37 ^{ab}	102.52 ^{b}	101.91 ^{b}	101.83 ^{b}	0.826
Feed conversion ratio (g/g)							
1-21 day	1.57 ^{ab}	1.58 ^{ab}	1.51 ^{b}	1.71 ^{a}	1.63 ^{ab}	1.56 ^{ab}	0.023
22-42 day	2.01	2.07	2.05	2.13	2.10	2.02	0.017
1-42 day	1.86 ^{b}	1.90 ^{b}	1.87 ^{b}	2.00 ^{a}	1.95 ^{ab}	1.87 ^{b}	0.015

[1]garlic, thyme leaf, cinnamon, rosemary leaves, anise (B), alfalfa leaves, corn flower, senna leaves, absinthe (D), echinacea purpurea, water cress, absinthe, polygermander (E), alfalfa leaves meal, cinnamon, burdock root, licorice root (F), and thyme, caraway, carum copticum (G),$^{a-d}$ Means within the same row with no common superscripts differ significantly at $p \leq 0.05$.

Sampling and data collection

The birds live body weight and feed intake per pen were measured weekly. Feed conversion ratio was calculated on a pen weight basis. Mortality and dead bird weights were recorded daily. At 21 and 42 days of age two representative birds from each pen were slaughtered and carcass parameters including dressing percent, abdominal fat, relative weights of different parts of digestive tract and its accessory glands as well as relative weights of spleen and bursa of fabricius were determined. Blood samples from two randomly selected birds per pen were collected by wing-vein puncture, and sera were harvested from clotted blood by centrifugation at 2000 g for 15 min. Serum samples were kept in -24°C until measuring related parameters including cholesterol (CHL), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL). CHL, TG and HDL were measured by spectrophotometer using commercial Kits (Pars Azmoon) according to the manufacturer's protocols. VLDL values were calculated equal to TG values divided by 5.

At 6, 16 and 26 days of age, each bird received one dose of commercially Newcastle disease virus (NDV) vaccine. ELISA antibody titers against NDV were determined at 21, 31 and 42d of age using the IDEXX NDV Antibody Test Kit (IDEXX laboratories Inc., Westbrook, ME 04092) according to the manufacturer procedure.

Statistical analysis

Collected data were analyzed by General Linear Model (GLM) procedure of SAS (SAS User's Guide, 2001). Duncan's multiple-range test was used to detect the differences between treatments, and significance defined as a P value equal to or less than 0.05.

RESULTS AND DISCUSSION

Growth performance

The effect of different medicinal plants blends on body weight, daily weight gain, feed intake and feed conversion ratio are shown in Table 2. Live body weight and daily weight gain were significantly ($p < 0.05$) decreased in birds fed blend E containing diet in both 21 and 42 days as compared to control birds. The effects of supplemented diets on body weight were only observed in blend D fed birds, in which insignificant improvement of body weight by 7.32 and 4.34% were recorded at 21 and 42 days respectively. The lower growth performance in blend E fed broiler chickens could be due to the presence of polygermander in this blend. Hassani et al. (2008) showed that addition of polygermander to broiler diet caused poor growth performance. Also, hepatotoxic effect of polygermander has been reported in human studies (Starakis et al., 2006; Savvidou et al., 2007). Furthermore, variation in the manipulation conditions of commercially obtained herbs, particularly at drying process, may attribute to poor growth performance in birds fed certain herbal blends.

Table 3. Effect of medicinal plant blends on serum lipids concentration.

Medicinal plant blends[1]	Triglyceride	Cholesterol	HDL	LDL	VLDL
21 days of age					
C	119.89[a]	147.22	87.64	31.00	23.98[a]
B	117.62[ab]	136.11	67.85	44.74	23.53[ab]
D	92.61[b]	141.21	91.88	52.92	18.52[b]
E	44.32[c]	134.26	76.33	23.62	8.863[c]
F	115.34[ab]	147.22	92.35	37.86	23.07[ab]
G	96.59[ab]	150.00	89.05	50.25	19.32[ab]
SEM	6.21	3.72	9.42	5.83	1.24
33 days of age					
C	92.58	91.49[c]	56.57	16.40	18.52
B	122.73	116.60[ab]	65.33	26.74	24.55
D	91.78	95.84[bc]	54.98	22.50	18.36
E	87.17	107.00[abc]	52.93	33.52	17.44
F	112.27	121.11[a]	70.92	21.54	22.46
G	107.97	120.96[a]	63.37	40.06	21.60
SEM	5.50	3.69	2.78	3.03	1.10
42 days of age					
C	73.87	121.30[ab]	78.21	28.32[ab]	14.78
B	68.75	136.58[ab]	73.97	54.29[a]	13.75
D	70.46	125.00[ab]	80.10	28.25[ab]	14.09
E	63.07	126.39[ab]	78.84	40.34[ab]	12.61
F	96.02	120.83[b]	81.98	19.42[b]	19.21
G	111.93	145.37[a]	60.94	54.91[a]	22.39
SEM	6.47	3.19	4.17	4.47	1.29

[1]Control (C), garlic, thyme leaf, cinnamon, rosemary leaves, anise (B), alfalfa leaves, corn flower, senna leaves, absinthe (D), echinacea purpurea, water cress, absinthe, polygermander (E), alfalfa leaves meal, cinnamon, burdock root, licorice root (F), and thyme, caraway, carum copticum (G), [a, b & c] Means within the same column with no common superscripts differ significantly $p \leq 0.05$

No differences in feed intake were observed in birds fed the blend of medicinal plants as compared to control birds, whereas feed intake in birds fed blend B was higher than those fed blend E in 1 to 21 and 1 to 42 days phases, and blend F fed chickens in 21 to 42 days rearing phases (Table 2). There was no significant difference in feed conversion ratio between chicks fed medicinal plant blends and that of those fed the control diet, however, feed conversion ratio of chicks in blend D treatment was significantly ($p < 0.05$) lower than that of blend E fed chickens.

Serum lipids

Serum cholesterol concentration was not affected by experimental treatments at day 21. At day 33, chickens fed blends B, F and G containing diets had higher cholesterol level than that of control birds (Table 3). Reduction of serum triglyceride and VLDL were also recorded at 21 d of age due to blends D and E supplementation when compared to control chickens. The HDL and LDL concentration were not affected by blends of medicinal herbs used in this trial (Table 3). Unexpectedly, herbal blends used in this study did not show any cholesterol lowering effect. In contrast, elevation of this metabolite was observed in the individual cases of certain experimental treatments. In contrast with our results, the cholesterol lowering effects of some phytobiotics have been reported by earlier researchers (Al-Kassie and Jameel, 2009; Kermanshahi and Riasi, 2006). Unaffected HDL concentrations and TG and VLDL lowering activities exhibited by blends B and D at day 21 are in agreement with results reported by Taimorizadeh et al. (2008) who indicated that extracts derived from oregano and garlic could decrease TG in broilers, bat HDL failed to respond to these treatments. Several mechanisms are proposed regarding the phytogenics effects on bird's lipid metabolism. As suggested by Qureshi et al. (1983), suppressed activities of enzymes

Table 4. Effect of medicinal plant blends on antibody titer (Log10) against NDV and relative weights (% of live body weight) of immune organs in broiler chickens.

	Medicinal plant blends[1]						
	C	**B**	**D**	**E**	**F**	**G**	**SEM**
Antibody titer							
21 day	2.524	2.368	2.391	2.465	2.417	2.377	0.044
33 day	2.583^{c}	3.058^{a}	3.260^{a}	2.269^{d}	2.957^{ab}	2.733^{bc}	0.090
42 day	3.359^{c}	3.057^{e}	3.210^{d}	3.623^{a}	3.648^{a}	3.517^{b}	0.045
Spleen							
21 day	0.094^{b}	0.104^{ab}	0.094^{b}	0.090^{b}	0.124^{a}	0.099^{b}	0.004
42 day	0.13^{b}	0.13^{b}	0.14^{ab}	0.12^{b}	0.17^{a}	0.10^{b}	0.006
Bursa of fabricius							
21 day	0.20^{ab}	0.26^{a}	0.22^{ab}	0.24^{ab}	0.24^{ab}	0.18^{b}	0.009
42 day	0.158	0.124	0.131	0.169	0.150	0.158	0.007

[1]Control (C), garlic, thyme leaf, cinnamon, rosemary leaves, anise (B), alfalfa leaves, corn flower, senna leaves, absinthe (D); echinacea purpurea, water cress, absinthe, polygermander (E), alfalfa leaves meal, cinnamon, burdock root, licorice root (F), and thyme, caraway, carum copticum (G), [a, b, c & d] Means within the same row with no common superscripts differ significantly $p \leq 0.05$.

involved in lipid metabolism including hepatic 3-hydroxy-3-methylglutaryl-CoA reductase, cholesterol 7a-hydroxylase, and fatty acid synthetase and in representative pentose-phosphate pathway, likely are the ways in which these treatments exert their effects on lipid metabolism.

Immune responses

There were not any significant effects of medicinal plants supplementation on anti-NDV antibody titer at day 21whereas, all medicinal plant blends, except for the G blend, increased ($p < 0.05$) antibody titer against NDV at day 33 and highest antibody titers were belonged to the groups fed blends B and D containing diets (Table 4). At the last measuring point (Day 42), addition of F, E and G blends to the broiler diets also resulted in higher ($p < 0.05$) antibody titers against NDV in compare to the control birds However, antibody titer was reduced in the blends B and D received chickens. Beneficial effects of blend F on immune responses also was concurrent with higher ($p < 0.05$) relative weights of spleen at both 21 and 42 days as compared to control chickens (Table 4).

Most consistent enhancements of immune response parameters were recorded in blend F receiving broilers. This may be due to antimicrobial and immunomodulatory properties of plants used in this blend including alfalfa, liquorice root, great burdock, and cinnamon. Polysavone (natural extract of alfalfa) has been known for its potent immuno-stimulatory effects that could increase antibody titer against NDV and relative weight of spleen in broilers (Dong et al., 2007). Furthermore, in the experiment conducted by Berezin et al. (2008) saponin extracted from liquorice accompanied by antigen of *Eimeria tenella* made a marked protective effect against this pathogen in broilers.

Carcass characteristics and organs weight

The effect of medicinal plant blends on carcass characteristics and organs weight is shown in Table 5. None of the medicinal plant blends could affect carcass yield at day 21 when compared to control. However, carcass yield for chicks in group D was higher ($p < 0.05$) than that of chicks in group G. At day 42, chicks in group F showed less carcass yield than control and D groups. Blend D numerically improved carcass yield values at both 21 and 42 days of age. This could be related to relatively better growth rate caused by this treatment and to decreased weights of inner organs, in particular, different sections of small intestine.

Supplementation of broiler diets with medicinal plant blends did not altered liver and gizzard weight as compared to control birds. Feeding diets containing blend F in broiler chickens increased pancreas weight in comparison to control and blend B receiving groups at 42 days of age. All additives except B and D showed a trend to increase abdominal fat deposition at day 42 whereas there were no considerable changes in this parameter induced by the experimental treatments at day 21. Similar to this finding, increased abdominal fat in broilers fed by thyme leaves, which was a component of blend B in our experiment, is previously reported (Ocak et al., 2008).

Table 6 shows the influence of our experimental treatments on relative weight and length of small intestine parts in broilers at day 21. The relative weight of

Table 5. Effect of medicinal plant blends on carcass yield and organs weight (% of live weight).

	Medicinal plant blends[1]						
	C	**B**	**D**	**E**	**F**	**G**	**SEM**
Carcass yield							
21day	56.38^{ab}	55.34^{ab}	57.50^{a}	56.30^{ab}	56.27^{ab}	54.77^{b}	0.332
42day	63.743^{a}	62.688^{ab}	64.138^{a}	63.196^{ab}	61.283^{b}	62.560^{ab}	0.313
Liver							
21day	3.03^{ab}	3.49^{a}	2.93^{b}	3.27^{ab}	3.47^{a}	3.22^{ab}	0.071
42day	2.469	2.419	2.439	2.298	2.536	2.273	0.049
Pancreas							
21day	0.47	0.43	0.45	0.45	0.50	0.47	0.010
42day	0.278^{bc}	0.259^{c}	0.275^{bc}	0.284^{abc}	0.323^{a}	0.309^{ab}	0.006
Proventriculus							
21day	0.65	0.60	0.56	0.64	0.64	0.60	
42day	0.388	0.424	0.393	0.431	0.400	0.429	0.008
Gizzard							
21day	2.92^{ab}	2.81^{b}	2.82^{b}	3.24^{a}	2.89^{ab}	2.72^{b}	0.057
42day	1.706	1.789	1.794	1.848	1.783	1.774	0.025
Abdominal fat							
21day	1.00^{ab}	1.02^{ab}	1.11^{a}	1.06^{ab}	0.78^{b}	1.26^{a}	0.042
42day	1.09^{d}	1.84^{a}	1.33^{bcd}	1.23^{cd}	1.65^{abc}	1.69^{ab}	0.068

[1]Control (C); garlic, thyme leaf, cinnamon, rosemary leaves, anise (B), alfalfa leaves, corn flower, senna leaves, absinthe (D), echinacea purpurea, water cress, absinthe, polygermander (E), alfalfa leaves meal, cinnamon, burdock root, licorice root (F), and thyme, caraway, carum copticum (G), [a-d] Means within the same row with no common superscripts differ significantly $p \leq 0.05$.

Table 6. Effect of medicinal plant blends on relative weight (% of body weight) and relative length (% of small intestine length) of intestine parts in broiler chickens.

	Medicinal plant blends[1]						
	C	**B**	**D**	**E**	**F**	**G**	**SEM**
Duodenum weight							
21day	1.45^{a}	1.32^{ab}	1.14^{b}	1.44^{a}	1.40^{a}	1.25^{ab}	0.032
42day	0.610^{b}	0.588^{b}	0.639^{b}	0.690^{ab}	0.758^{a}	0.613^{b}	0.017
Jejunum weight							
21day	2.47^{a}	1.91^{c}	2.04^{bc}	2.25^{ab}	2.27^{ab}	2.05^{bc}	0.045
42day	1.248	1.278	1.238	1.364	1.401	1.238	0.026
Ileum weight							
21day	1.79^{a}	1.57^{c}	1.60^{bc}	1.76^{ab}	1.69^{abc}	1.70^{abc}	0.025
42day	0.96	1.09	0.96	1.11	1.10	1.04	0.022
Cecum weight							
21day	0.58^{ab}	0.57^{ab}	0.50^{b}	0.61^{a}	0.62^{a}	0.58^{ab}	0.013
42day	0.39	0.38	0.37	0.42	0.44	0.40	0.010
Duodenum length							
21day	0.183	0.181	0.168	0.188	0.175	0.173	0.003
42day	0.171	0.165	0.166	0.173	0.174	0.170	0.002

Table 6. Continued.

Jejunum length							
21day	0.41	0.40	0.41	0.39	0.40	0.40	0.003
42day	0.400^{ab}	0.398^{ab}	0.399^{ab}	0.383^{b}	0.403^{a}	0.403^{a}	0.003
Ileum length							
21day	0.41	0.42	0.42	0.42	0.43	0.43	0.003
42day	0.430	0.436	0.436	0.445	0.425	0.428	0.003

[1]Control (C); garlic, thyme leaf, cinnamon, rosemary leaves, anise (B), alfalfa leaves, corn flower, senna leaves, absinthe (D), echinacea purpurea, water cress, absinthe, polygermander (E), alfalfa leaves meal, cinnamon, burdock root, licorice root (F); and thyme, caraway, carum copticum (G), $^{a, b, c \& d}$ Means within the same row with no common superscripts differ significantly $p \leq 0.05$.

duodenum in group D, and relative weights of jejunum and ileum in blends B and D groups were lower than those of control group ($p < 0.05$). At day 42, the inclusion of 10 g/kg blend F increased duodenum weight ($p < 0.05$). The broilers small intestine length was unaffected by blends of medicinal plants administered in this study

In conclusion, while the poultry industry is looking for the safe and efficient growth enhancers, some of the used blends such as blend D that contained alfalfa, senna, corn flower and absinthe may be a proper candidate to fulfill the demand of poultry industry in search for safe and efficient growth enhancers, however, further studies are necessary to absolute judgment.

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