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Original Article

Effects of Medicinal Plants Extract with Antibiotic Free Diets on Broilers Growth Performance and Incidence of Muscles Abnormalities

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■Keywords

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ABSTRACT

The objective of this research was to investigate the effects of a natural herb extract[1] (HE) on the general growth performance and the incidence of muscle abnormalities (white striping and wooden) of broiler chicks. A total of 504 one-day old chicks (Ross 500) were used in the experiment and randomly partitioned into 3 treatments with 7 replicates in each (24 chicks per replicate). Three experimental diets (C: regular feed; T1 and T2 (supplemented with HE at levels of 0.20 and 0.30 ml/L, respectively). The herbal extract was supplemented in drinking water. Feed intake, body weight and feed conversion ratio were monitored at days 14, 28 and 42 of the feeding trial. Moreover, the yield of visceral organs, carcass cuts, and the incidence of muscle abnormalities were evaluated at different ages of slaughter (34, 41and 48 days). Results showed that weights, feed conversion ratios (FCR) and dressing percentages (DP) increased (p<0.05) in birds supplemented with herb extract compared to control birds. However, HE supplementation had no significant effects on carcass cuts and some visceral organs. Meat quality was improved by HE supplementation as the incidence of white stripping (WS) and WS plus wooden meat cases dramatically reduced, in particular at 34 days of age, day of slaughter. It can be concluded that feeding the herbal extract has significant positive effects on broilers general performance, feed efficiency and meat quality.

INTRODUCTION

The use of antibiotics is restricted in many parts of the world. However, 80% of raised livestock were fed some kind of antibiotics or growth promoters. Recently, herbs extract were fed as nontraditional feed additive to replace the growth promoters and antibiotics Lee *et al.*, 2001). Herbal extracts were used in the extensive livestock system to improve animals' general performance (Abo Omar *et al.* 2016; Bakhiet & Adam, 1995; Gill *et al.* 2002; Horton *et al.*, 1991; Janssen, 1989; Manzanilla *et al.*, 2001; Skrabka *et al.*, 1997; William & Losa, 2001). The positive effects of herb extracts were due to the improvement of feed intake, digestibility, activation of the immune system and the anthelmintic actions. Fenugreek was reported to have positive effects on the digestive system (Raju *et al.,* 2004) and to be rich in protein, total carbohydrates and minerals, Gupta *et al.* (1996)[10].

Kolacz *et al.* (1997) reported that the excessive growth of gastrointestinal tract, harmful microorganisms and inflammations were minimized by feeding chamomile flowers. It was found that Nettle (*Urtica dioica*) which is composed of starch, gum, albumen,

[1] Herb extract (HE) is a mixture of pure honey with an extract of several medicinal plants: fenugreek (Tigonella foenum graecum), chamomile (Anthemis ecutita), nettle (Urtica dioica), thyme (Thymusvulgaris), mint (Menthola), black seed (Nigella sativa).

sugar, histamine, acetylcholine, choline, and serotonin is fed to animals. Thyme (*Thymus vulgaris*) is also used for medicinal purposes through its components (thymol, 40% and carvacrol, 15%) (Mikaili *et al.*, 2010). Mentha species of the family *labiatae* have antiseptic properties and improve digestibility which have beneficial effects on digestion (Foster & Duke, 1999). Studies on the pharmacological action of active plant substances or herbal extracts in animal nutrition is relatively limited.

Currently, there is a great demand on broiler meat and there is a great success in increased growth rate in order to optimize the production of this meat. The improvement of growth rate resulted in high incidence of muscle abnormalities. The most recent and emerging muscle abnormalities are white striping (WS) and wooden abnormalities (WA) (Petracci *et al.*, 2019). In addition, visible/near infrared spectroscopy has been investigated to differentiate between normal and white striped meat (Zaid *et al.*, 2019)

Mudalal (2019) reported that the total incidence of white striped breast fillets under commercial conditions in different genotypes of broilers was very high and reached up to 61.3%. Considering the effect of genotype, the results showed that high-breast yield hybrids exhibited a higher overall incidence of WS compared with standard-breast yield hybrids (Petracci *et al.,* 2019).

The objective of this research was to investigate effects of a blend of natural herb extract on growth performance and incidence of muscles abnormalities of broilers reared under antibiotics free rations.

MATERIALS AND METHODS

Experimental animals and design

A total of 504 one-day old chicks (Ross 500) were purchased from a local hatchery. At the experimental site, chick's initial weights were recorded and randomly housed in wood shavings covered floor pens. Chicks were divided into 3 treatments with 168 birds in each pen and to 7 replicates per treatment in a complete random design. Continuous lighting was provided throughout the experiment. The ambient temperature was gradually decreased from 32 °C on day 1 to 7 °C to 24 °C on day 21 and was then kept constant.

The diet's ingredients components fed and their chemical analysis are presented in Table 1. The starter diets were fed for the first three weeks and the grower diet was fed for the remainder of the trial (48 days).

Chicks were assigned to the control diet (control, C) which is similar to regular broiler starter diets. While the experimental treatments T1 and T2 birds were fed the diets as in control but was supplemented with the herb extract at levels of 0.2 and 0.3ml/L in drinking water. Birds diets were formulated to meet the NRC (1990) recommendations. Feed intake was recorded in a weekly basis, by deducting the amount of feed that remained in the feeders at end of the week from the amount offered. Individual bird weight was also recorded in a weekly basis. Birds' mortalities within the five days were replaced, afterwards, mortality was recorded as it occurred and dead chick weights were determined where possible.

Table 1 – Composition of the basal diets fed to broilers in feeding trial, g/kg.

Ingredient	Starter	Grower
Yellow corn	560	620
Soybean meal	360	306
Oil	40	40
DCP1	15	12
Limestone	15	15
NaCl	3.5	3.5
Premix	5	5
DL-methionine	1	1
L- lysine	0.5	0.5
Calculate analysis		
Crude protein	220	200
Lysine	110	110
Methionine	55	56
Calcium	100	110
Available P	46	47
ME, MJ/ kg ration	704	718

[1]Dicalcium phosphate. [2] Vitamin premix/kg diet: vitamin A, 12,000 IU; vitamin D3,1500 IU; vitamin E, 50 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 0.03 mg; niacin, 25 mg; Ca-D-pantothenate, 12 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apo-carotenoic acid ester, 2.5 mg; choline chloride, 400 mg.

Body weight (BW), feed intake (FI) and feed conversion ratios (FCR) were assessed on days 14,28 and 42 or during the age ranges of 1- 14, 14 - 28 and 28 - 42 days. FCR was calculated as the following: FCR= average feed consumed/average live weight

Visceral organs and carcass cuts

At 42 d of age, eight birds per replicate were slaughtered through cutting carotid arteries and partial slicing of the neck by a manual neck cutter. Carcass yield was calculated by dividing eviscerated weight by live weight. Visceral organs (liver, gizzard, heart, proventriculus, small intestine, cecum) and abdominal fat were weighed and calculated as a percentage of live body weight and also carefully examined to detect any pathological lesion or damage. Carcass parts were measured as percent of carcass weight.

Assessment the incidence of muscle abnormalities

After about 8 hr of post mortem, breast fillets from all treatments and replicates were collected to assess the incidence of muscle abnormalities. Based on previously described criteria by Kuttappan *et al.* (2012b) and Sihvo *et al.* (2014), the muscle abnormalities were classified into four categories (Normal, white striping, wooden, and white striping/wooden). Breast fillets were labeled as normal (N) when there were no white striations or hardened areas over the surface. When breast fillet exhibited white striations (thin to thick striations) on the surface, it was labeled as white striped fillet (WS). In the case of presence of pale ridge-like bulges and diffuse hardened areas, breast fillet was labeled as wooden breast (WB). Finally, when a fillet was affected by both muscle abnormalities white striping and wooden breast, it was labeled as WS/WB.

Statistical analysis

The results of the study were analyzed using the ANOVA (GLM procedure SAS statistical analysis software, 2002). It was used to evaluate the effect of herbs extract (two levels of inclusion) and replicates on the growth performance, feed conversion ratio, and the incidence of muscle abnormalities. Duncans test was employed to separate the mean value in the case of presence of statistical differences ($p>0.05$). Pearson correlation was used to test the relationship between pairs of continuous variables (*i.e.*, feed con-version ratios, carcass and visceral organ variables).

RESULTS

Feed intake

Results showed that the feed intake was significantly increased in group T1 and T2 if compared to control group C at age 1-14d (35.52 and 36.09 *vs.* 34.74 g/d, $p<0.05$) and age 1-42d (118.29 and 118.03 *vs.* 117.10, $p<0.05$). The HE significantly increased ($p<0.05$) feed intake when fed at 0.2 and 0.3 ml/L compared to the control birds during the entire feeding trial (Table 2).

Body weight

Birds supplemented with the two levels of the extract gained more ($p<0.05$) weight at all growth periods compared to birds consuming the regular broiler diet (Table 2).

Body weight significantly increased in birds of group T1 and T2 compared to the control at ages 1-14 d (456.4, 481.0 *vs.* 434.4 g, $p<0.05$), 1-28d (1462.06, 1513.27 *vs.* 1412.09g, ($p<0.05$) and 1-42d (2574.3, 2671.4 *vs.* 2486.2 g, ($p<0.05$) Table 2.

Feed conversion ratio

HE had positive effects on FC ratio. FCR significantly improved in birds of groups T1 and T2 compared to the control group birds at age of 28 d (1.530, 1.476*vs.* 1.578, $p<0.05$). However, HE had variable effects on FCR where T2 birds had better feed efficiency compared to T1 birds (1.476 *vs.* 1.530, $p<0.05$). At 42 d of the feeding trial, FCR in birds of T1 and T2 was similar but significantly lower compared to that of control birds (2.100, 2.183 *vs.* 2.249, $p<0.05$). For the entire feeding period, the FCR that most improved was in birds of T2 followed by T1 and control (1.667 vs. 1.734, 1.785, $p<0.05$), Table 2.

Visceral organs

Visceral organs were expressed as percentages of live weights. HE had no effects on abdomen fat, pancreas, and hear. However, there was a numerical difference

Table 2 – Effect of herb extract (HE) supplementation on performance indices of broilers at different ages. The basal diet (control, C) which is similar to regular broiler starter diets. While the experimental treatments T1 and T2 birds were fed the diets as in control but was supplemented with the herb extract at levels of 0.2 and 0.3ml/L in drinking water.

		C	T1	T2	*p* value
Feed intake	1 - 14 d	34.75^{b}	35.52^{a}	36.09^{a}	0.002
	14 - 28 d	110.19	109.93	108.76	0.07
	28 - 42 d	172.29	173.47	173.27	0.358
	1 - 42 d	117.10^{b}	118.29^{a}	118.03^{a}	0.001
Body weight	14 d	434.48^{c}	456.35^{b}	481.86^{a}	0.000
	28 d	1412.09^{c}	1462.06^{b}	1513.27^{a}	0.000
	42 d	2486.20^{c}	2574.30^{b}	2671.40^{a}	0.000
Feed conversion ratio	1 - 14 d	1.11^{a}	1.09^{ab}	1.04^{a}	0.005
	14 - 28 d	1.57^{a}	1.53^{b}	1.47^{c}	0.000
	28 - 42 d	2.24^{a}	2.18^{b}	2.10^{b}	0.020
	1 - 42 d	1.78^{a}	1.73^{a}	1.66^{b}	0.000

Different letters in the same row indicate significant differences ($p<0.05$).

among the tested parameters. Proportions of liver to live weight was lowest in T1 birds compared to T2 and control birds (2.64 *vs*. 2.96, 2.92%, $p<0.05$). However, the two levels of the extract (T1 and T2) reduced ($p<0.05$) the percentages of gizzard, crop and intestine percentages compared to that in control birds (Table 3).

Table 3 – Relative percentages of visceral tissues and carcass cuts of broilers supplemented with herb extract (HE). The basal diet (control, C) which is similar to regular broiler starter diets. While the experimental treatments T1 and T2 birds were fed the diets as in control but was supplemented with the herb extract at levels of 0.2 and 0.3ml/L in drinking water.

Parameter	C	T1	T2	*p* value
Pancreas	0.19	0.17	0.19	0.124
Abdonin fat	1.28	1.14	1.30	0.245
Legs	12.34	4.65	3.79	0.371
Bursa	0.12^{b}	0.11^{b}	0.14^{a}	0.000
Crop	0.72^{a}	0.58^{ab}	0.65^{b}	0.005
Gizzard	2.41^{a}	1.82^{b}	2.10^{b}	0.000
Spleen	0.19^{b}	0.17^{b}	0.20^{a}	0.010
Liver	2.92^{a}	2.64^{b}	2.96^{a}	0.000
Heart	0.57	0.55	0.55	0.391
Intestine	5.67^{a}	4.88^{b}	4.92^{b}	0.000
Neck	7.17^{a}	6.28^{b}	7.01^{a}	0.000
Breast	41.53	38.20	41.64	0.111
Wings	9.18^{a}	8.39^{b}	9.14^{a}	0.001
Drum stick	11.77	11.18	11.71	0.242
Viscera	14.87^{a}	12.96^{b}	13.74^{b}	0.000
Live weight	3.20^{a}	2.97^{ab}	3.13^{b}	0.040
Dressing %	78.0^{b}	82.0^{a}	77.5^{b}	0.034

Different letters in the same row indicate significant differences ($p<0.05$).

Dressing percentages

The HE had significant ($p<0.05$) effects on dressing proportions. The dressing percentages of T1 birds was higher compared to T2 and the control birds (82, *vs*. 77.5, 78%, $p<0.05$), Table 3.

Carcass cuts

Most of carcass cuts were not affected by the supplementation of the HE, however, the percentages of neck and wing significantly reduced in T1 compared to these in T2 and the control birds (Table 3).

Lymphoid organs

The effects of treatments on immune related tissues are shown in Table 4. Spleen and bursa percentages were affected ($p<0.05$) by HE supplementation. The bursa of T2 birds had the highest percentages compared to T1 and the control birds (0.145 *vs*. 0.11, 0.12%, $p<0.05$). A similar trend was observed for spleen percentage where T2 birds had more spleen weight compared to T1 and the control birds (0.20 *vs*. 0.17, 0.19%, $p<0.05$), Table 3.

Muscle quality

The incidence of muscle abnormalities (white striping or white striping combined with wooden) at different ages of slaughter (34, 41, and 48 d) was shown in Table 4. Our study revealed that the addition of herb mix had great ($p<0.05$) impact on the incidence of muscle abnormalities, and this effect was very clear at 34 days of age. In this context, the incidence of white striping combined with wooden abnormalities in group T1 and T2 was 0% while in control group (C) it was 35.2%. At slaughter age, the incidence of white striping in group T1 and T2 was lower than in the control group (C) (28.1 and 29.8 *vs*. 38.5%) respectively. At higher age of slaughtering (41 and 48 d), the effect of herbs extract on the incidence of muscle abnormalities was less if compared to 34 days of age. Breast fillets at age 41 and 48 did not show any normal cases in all treatments. The incidence of white striping in group T1 and T2 at age 41 (32.8 and 19.5 *vs.* 39.2) and age 48 (23.5 and 25.5 *vs*. 33%) was lower than the control group.

DISCUSSION

Feed intake

The HE had a significant effect on feed intake of treated chicks. The highest intake was in birds consuming the HE at the two levels compared to the control birds. Similar findings were observed in previous researches where feed intake was higher in broilers fed 0.2 ml/L of medicinal herb extract (Abo Omar *et al.,*

Table 4 – Percentages of normal, white striping (WS) and WS plus wooden meat abnormalities of broilers supplemented with herb extract (HE) at different ages. The basal diet (control, C) which is similar to regular broiler starter diets. While the experimental treatments T1 and T2 birds were fed the diets as in control but was supplemented with the herb extract at levels of 0.2 and 0.3ml/L in drinking water.

	C-d 34	T1-d 34	T2-d 34
Normal	26.4[b]	71.9[a]	70.2[a]
white striping	38.5[a]	28.1[b]	29.8[b]
white striping + wooden meat	35.2[a]	0.0[b]	0.0[b]
	C-d 41	T1-d 41	T2-d 41
Normal	0	0	0
white striping	39.2[a]	32.8[a]	19.5[b]
white striping + wooden meat	60.8	67.2	80.5
	C-d 48	T1-d 48	T2-d 48
Normal	0	0	0
white striping	33.0	23.5	25.5
white striping + wooden meat	67.0	76.5	74.5

Different letters in the same row indicate significant differences (p<0.05).

2016). However, anise seeds had negative effects on feed intake when fed at levels from 1 to 10 g/kg ration (Yazdy *et al.* (2014). The positive effects of HE on feed intake presented in this research could be related to improvement of appetite (Raju *et al.* 2004; Abo Omar *et al.*, 2016).

Body weight

The two supplemental levels of the HE (0.2 and 0.3 ml/L) improved broiler growth. The same effect of medicinal plant extract on broilers body weight were reported by Abo Omar *et al.* (2016). Our findings were in disagreement with previous research when anise seeds had no effect on broilers growth Yazdy *et al.* (2014). The essential fatty acids (linolenic and linoleic) in black seeds might explain our results regarding the improvement in broilers growth (Murray *et al.*, 1991). A dose of 5 g/kg of thyme had significant improvement on broilers weight and feed conversion ratio (Toghyani *et al.*, 2010). Similar trend was reported by Najafi & Turki (2010) where thyme-included diet had significantly increased broilers body weight and feed conversion ratio, however, negative effects of thyme were observed on broilers performance (Demir *et al.*, 2008; Tekeli *et al.*, 2006).

Feed conversion ratio

Best feed conversion ratio was observed in broilers fed high level of HE (0.3 ml/ L) levels. This finding agrees with previous research where medicinal herb extract had significant improvement in feed efficiency, 8 to 11 (Abo Omar *et al.,* 2016). Similar results were reported with anise seeds when fed to broilers (Yazdy *et al.,* 2014). The suppression of gram-negative bacteria and clostridium with other growth depression agents along with the improvement of feed nutrient digestion and absorption can explain the better general performance and the feed conversion ratio (Abd El –Latif *et al.,* 2004; El-Gendy *et al.,* 1996; Ghazalah & Ibrahim, 1996). The positive advantages HE on animals' performance could be achieved through the antioxidants, antibacterial, antifungal and antiprotozoal agents (Leung & Foster, 1996). The alteration of some physiological and chemical functions of the digestive tract by some plant metabolites such as isoprene derivatives, flavonoids and glucosinolates might cause the observed effects (Baratta, *et al.* 1998; Horton *et al.*, 1991; Jamroz *et al.*, 2003). Body weight gain, feed conversion ratio and protein efficiency ratio significantly improved for chicks fed diets supplemented with fenugreek at rate of 0.5% or 1.5% as compared to the control diet (Elbushra, 2012).

Similarly, supplementation of chamomile flowers at level of 2.5 kg/ton of broiler diet improved growth performance and feed conversion (Abaza *et al.,* 2008). There is a debate on the real effects of nettle (a component of our plant extract) on broilers performance. The carvacrol in nettle has stimulatory effects on pancreatic secretions (Mansoub, 2011) by increasing the secretions of digestive enzymes more amounts of nutrients like amino acids can be digested and absorbed from the digestive tract and thereby improve carcass traits. Abedin *et al.* (2019) reported that the high amount of bioactive compounds in Chinese herbal medicine byproducts confer several nutritional and health benefits. Similarly, medicinal plant leaf meal improved broilers growth and feed conversion ratio through improvement of anti-oxidative capacity without negative effects on birds' health (Daramola, 2019).

Visceral tissues

HE had no effects on abdomen fat, pancreas, and heart. Proportions of liver weights to live weight was lowest in birds fed 300 ml/L of the extract. However, the two levels of the extract reduced the percentages of gizzard, crop and intestine proportions. Abo Omar *et al.* (2016) reported similar results for broilers fed medicinal plant extract.

Dressing percentages

Feeds supplemented by the HE resulted in higher dressing proportions compared to the control birds. Similar trends were observed in previous research (Issa & Abo Omar, 2013; Abo Omar *et al.*, 2016), where medicinal plants improved dressing percentages. The dressing proportions in this research were relatively higher compared to what was reported as our feeding period was one week longer.

Carcass cuts

Most of the carcass cuts were not affected by the supplementation of the HE, however, percentages of neck and wing were significantly reduced. Similar results were reported previously (Abo Omar *et al.,* 2016; Issa & Abo Omar, 2013, Abo Omar *et al.,* 2016).

Lymphoid organs

Both bursa and spleen relative weights increased in birds supplemented with HE. Yazdy *et al.* (2014) reported that anise seeds had no effect on spleen but caused significant increase in bursa weight. However, medicinal herb extracts reduced the weights of spleen and bursa of broilers (Abo Omar *et al.*, 2016).

The low mortality and the good performance of broilers can be explained by the enhancement of the immune system as indicated by larger spleen and bursa weights.

Muscle quality

Inclusion of the herbs mix in the drinking water of chicks showed significant reduction in the incidence of muscle abnormalities (white striping and wooden) at 34 days of age, day of slaughter. Herbal mix that has been employed in this study contains several functional ingredients such as thymol, carvacrol, antioxidants, serotonin, etc as well as many of essential minerals (calcium, phosphorus, iron, zinc and magnesium). In general, the etiological origin of these muscle abnormalities is still unclear but there are several hypotheses that can explain the potential etiology. It was found that the incidence of muscle abnormalities was commonly in fast-growing and high-breast development in modern broiler hybrids. These broiler hybrids were characterized by hypoxia which was attributed to low density of blood capillaries and insufficient oxygen and nutrients supply (Sihvo *et al.* 2018; Boerboom *et al.,* 2018). Another hypothesis, the tremendous growth in breast muscle may generate extra compression over the artery of pectoral muscle which contributes to more reduction in oxygen and nutrients supply to muscle. Several researchers found that the severity of histological lesions in white striped and wooden breast increases by moving from the inner core of pectoralis major muscle towards the surface of muscle which supports the previous hypothesis (Clark & Velleman, 2016; Soglia *et al.,* 2016; Baldi *et al.*, 2018). At low age of slaughter (in our case 34 days), the impairment in the oxygen and nutrients supply due to growth may be very low which. Therefore, the birds may be able to supply of functional ingredients in the mix herbs to their muscle which contributed to reduce the incidence of white striping and wooden muscle abnormalities. On the hand, at high ages of slaughter (41 and 48 days), the muscles growth is usually very high and the impairment of oxygen and nutrients supply is sharply affected. Therefore, the functional nutrients in herb mix may not reach the affected muscles, this may explain why at high ages of slaughter, there was no effect of herbs mix on the incidence of white striping and wooden muscle abnormalities. In conclusion, the HE had several advantages when supplemented to broilers. Significant improvement in body weights, FCR and dressing percentages were achieved. Application of HE reduced the incidence of muscle abnormalities then broiler meat quality. Our results were comparable to Cobb 500 standards.

CONFLICT OF INTERESTS

The authors declare no potential conflict of interest.

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