

Effects of Some Plant Extracts in Triticale-Based Broiler Diets on Growth Performance, *E.Coli* Counts in Intestine and Blood Parameters

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Abstract

This study was conducted to determine the effect of using plant essential oils in triticale-based broiler diets as alternative to antimicrobial growth promotors (AGPs) on growth, *E.coli* counts in small intestine and blood parameters. 720 male broiler chicks were randomly distributed to nine experimental groups with four replicates. The control group received the triticale-soybean meal basal diet. In the other treatment groups the basal diet was supplemented with one of the following; flavomycin, enzyme, flavomycin+enzyme, enzyme+thyme oil, enzyme+fennel oil, enzyme+bay leaf oil, enzyme+thyme oil+fennel oil and enzyme+thyme oil+fennel oil+bay leaf oil. Results showed that the weight gain and feed intake were not influenced by the treatments ($p>0.05$). The use of the combination of plant extracts significantly improved the feed conversion. Use of plant extracts in triticale-based broiler diets as an alternative to AGPs had no regular effect on *E.coli* count in small intestine segments ($p>0.05$). Feeding broiler chickens with the enzyme+thyme oil supplemented diets increased eritrocyte level in serum ($p<0.05$), but hemoglobin, leucocyte and hematocrit level in serum were not significantly changed ($p>0.05$) by the treatments. In conclusion, these plant extracts can be also used with enzyme in triticale-based broiler diets as alternative to AGPs.

Keywords: broiler chickens, enzyme, flavomycin, plant extracts, triticale

1. Introduction

After banning the use of AGPs in poultry diets, researchers have concentrated on finding alternatives to them for broiler diets that based on corn-soybean meal and other cereals-soybean meal.

Triticale is a cereal product which is not suitable for wheat agriculture, can be grown in barren lands with insufficient rainfall compared to cereals such as wheat, barley and oat. Although the protein content of triticale varies significantly according to its types, it is generally higher in wheat and rye which are considered as its parents.

Maurice et al. (1989) [1] showed that the use of triticale of 58 to 69% in broiler diets did not have any negative impacts on the weight gain and feed intake. The polysaccharides in triticale that are soluble in water restrict the use of triticale in broiler diets. The negative effects of nonstarch polysaccharides (NSPs) are resolved with the addition enzymes to the diets. Johson and Eason (1988) [2] reported that triticale of 35% without any enzymes and of 60% with enzymes can be used in broiler diets. Researchers have determined that the water-soluble arabinoxylanes cause an increase in viscosity of digesta in small intestine [3] and stimulate the proliferation of microflora and microbial function [4]. The negative effects of NSPs can be prevented by adding enzymes to diets.

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The AGPs used as growth factor in poultry diets are materials created by microorganisms (bacteria, mushrooms, green plants) and preventing and destroying other microorganisms. Despite these positive effects, the use of antibiotics as growth factors is prohibited in the EU for ensuring food security that has become a top issue on the agenda in recent years with legal regulations. That is because the pathogen microorganisms become resistant to the AGPs if they are used for long periods and such antibiotics leave residues on the animal products. It is clear that the poultry performance will decrease with the prohibition of the AGPs. Thus, the studies on the use of feed additives separately or in combinations have been accelerated in recent years, which can be an alternative to the AGPs. These additives include primarily probiotics, prebiotics, organic acids and plant extracts.

New kinds of commercial feed additives in the form of powder and extracted essential oil originated from plants considered to be healthy, have antimicrobial and stimulatory activity in digestive system [5, 6]. There are different pure or mixed plant extracts that are used commercially in animal nutrition for their positive effects on growth and microorganisms. It is a well-known fact that the phenolic composites (caffeic, cinnamic, ferulic acid, gallic acid, thymol and eugenol), organic acids (benzoic, sorbic, citric, acetic acids) and essential oils have antimicrobial effects.

Nutritionists have conducted many studies on herbal feed additives as alternative to AGPs to manage to replace some herbal products to banned AGPs. However, there aren't enough studies to indicate the effects of new kinds of herbal oils such as fennel oil and bay leaf oil with enzymes in triticale-based broiler diets. The purpose of this study was to determine the effects of three plant extracts added to the triticale-based broiler diets with enzyme as alternative to AGPs on growth performance, *E.Coli* counts in the small intestine, and some blood parameters of male broiler chickens

2. Materials and methods

A total of 720, day-old male broiler chicks (Ross) were weighed individually on arrival then randomly assigned to one of nine dietary

treatments according to the random block design. Each treatment group consisted of 80 chicks with four replicates consisted of 20 chicks for each replicate. The chicks placed to the littered floor pens where they stayed during the 42 d of age. This experiment was conducted according to the guidelines of the University Animal Care and Use Committee. The temperature of the experimental unit was 32°C for the 1st week and then gradually decreased to 21°C for the 4th week. A continuous artificial lighting program was provided throughout the experimental period. Feed and water were supplied *ad libitum*. The composition of the basal diet is presented in Table 1.

The treatment groups (T) are established as follows:

T1 (Basal): Triticale-based diet

T2 (T+FLV): Basal+flavomycin

T3 (T+ENZ): Basal+enzyme

T4 (T+ FLV+ENZ): Basal+flavomycin+enzyme

T5 (T+ENZ+TO): Basal+enzyme+thyme oil

T6 (T+ENZ+FO): Basal+enzyme+fennel oil

T7 (T+ENZ+BLO): Basal+enzyme+bay leaf oil

T8 (T+ENZ+TO+FO): Basal+enzyme+thyme oil+fennel oil

T9 (T+ENZ+TO+FO+BLO): Basal+enzyme+thyme oil+fennel oil+bay leaf oil

Flavomycin was supplied by Kartal Chemistry Company (Istanbul, Turkey). The enzyme was Natuzyme (Bioproton Pty. Ltd., Australia) contained 4.200 IU cellulase/g, 7.500 IU xylanase/g, 700 IU α -amylase/g, 500 IU phytase/g and 50 IU pectinase/g as determined by the manufacturer. Thyme oil (*Thymus vulgaris*), fennel oil (*Foeniculum vulgare*) and bay leaf oil (*Laurus nobulis*) were taken from a local company. All these additives were used 1g/kg in diets.

Body weight gain was measured weekly. Feed intake was recorded weekly then feed conversion ratio (g:g) was calculated. 72 broiler chickens in total, including two from every replicate, were slaughtered on the 42nd d of the trial to determine the number of *E.coli* in small intestine segments and some blood parameters.

1 g of the intestine content was taken out by scraping the intestine wall of duodenum, jejunum and ileum sections of the small intestine to determine the number of *E. coli*. One gram of the content was diluted 1:9 (weight/volume) with physiological salt water (log₁₀). The samples of intestinal content were diluted from 10⁻¹ to 10⁻⁸.

Using 10^{-5} and 10^{-6} diluted samples *E.coli* was counted on MacConkey agar (MCA) and eosin

methylene blue (EMB) agar incubated at 37°C for 24h.

Table 1. The structure and chemical composition of the diets used in the trial, %

	Starter (0 to 14 d)	Grower (15 to 35 d)	Finisher (36 to 42 d)
Triticale	50.00	50.00	50.00
Corn	-	5.70	7.00
Soybean meal	35.30	27.30	32.5
Fish meal	5.30	7.0	-
Soybean oil	6.55	7.13	8.40
Dicalcium phosphate	1.00	1.00	0.70
Limestone	1.23	1.25	0.78
DL-Methionine	0.12	0.12	0.12
Sodium chloride	0.25	0.25	0.25
Vitamin and trace mineral premix	0.25	0.25	0.25
	Calculated contents		
Crude protein, %	23	21	19
Metabolisable energy, kcal/kg	3 100	3 200	3 300
Calcium, %	1.005	0.900	0.900
Available phosphorus, %	0.450	0.400	0.351
Methionine, %	0.58	0.501	0.36
Methionine+Cysteine, %	0.914	0.833	0.751
Lysine, %	1.48	1.046	1.00
Crude cellulose, %	4.025	3.778	3.407

2 ml of blood samples were taken from the jugular vein of the two broiler chickens from every replicate before slaughtering as stated above on the 42nd day of the trial. The blood was placed to the test tubes with EDTA and the tubes were processed with ABX Hemogram device and the hematocrit, hemoglobin, erythrocyte and leucocyte were determined.

Data were analyzed in accordance with the variance analysis by using SPSSWIN (1994) [7]. The significant differences between the groups' means were determined according to Duncan (1955) procedure [8].

3. Results and discussion

No significant differences were found regarding the body weight and feed intake at the end of the trial. The combined use of thyme oil and fennel oil or thyme oil, fennel oil and bay leaf oil in the broiler diets significantly improved the feed conversion when compared to the diet supplemented only with enzyme (Table 2). There was not any significant difference in the groups from day 0 to 42 in terms of mortality ($p>0.05$). The results regarding the number of the *E.coli* in the small intestine are given in Table 3. The number of *E.coli* in the duodenum was found

to be significantly low in the group with the diet of enzyme and flavomycin and the group with the diet of enzyme and fennel oil compared to other groups ($p<0.05$). There are some similarities between the groups with the diet of enzyme and thyme oil+fennel oil, thyme oil+fennel oil+bay leaf oil and the diets of enzyme and flavomycin and the number was determined to be significantly low compared to the control group ($p<0.05$). The number of *E.coli* of the group with the diet of enzyme and bay leaf oil was found to be high compared to the groups with the diet of flavomycin, enzyme, enzyme+flavomycin, enzyme+fennel oil, enzyme+fennel oil+thyme oil and enzyme+fennel oil+thyme oil+bay leaf oil ($p<0.05$) and there were some similarities with the control group ($p<0.05$). The number of *E.coli* in the jejunum was found to be high in the group with the diet of enzyme, thyme oil, fennel oil and bay leaf oil and low in the control group and the group with enzyme+fennel oil and enzyme+fennel oil+thyme oil ($p<0.05$). The other groups were ranked between these groups ($p<0.05$). The number of *E.coli* in ileum was found to be significantly high in the group with the diet of flavomycin without enzyme and of enzyme+bay leaf oil ($p<0.05$). The number of *E.coli* in the group with the diet of flavomycin with enzyme was found to be significantly low ($p<0.05$).

Table 2. Performance results of the broilers at the end of 42 d trial

Treatments	Initial body weight, g	Body weight, g	Feed intake, g	FCR, g:g	Mortality, %
Control	49.6±0.19	2227.4±65.1	3818.9±67.2	1.76±0.03 ^{ab}	5.00±2.04
T+FLV	49.4±0.22	2211.1±43.4	3764.6±111.1	1.74±0.05 ^{ab}	0.00±0.00
T+ ENZ	49.5±0.27	2217.2±40.4	3856.7±91.3	1.78±0.01 ^a	5.00±2.04
T+ FLV+ENZ	49.9±0.30	2286.9±12.1	3813.3±14.4	1.71±0.01 ^{ab}	1.25±1.25
T+ENZ+TO	49.7±0.29	2233.1±49.8	3741.2±85.9	1.72±0.02 ^{ab}	3.75±2.39
T+ENZ+FO	49.8±0.35	2253.4±36.4	3781.9±42.4	1.72±0.03 ^{ab}	3.75±2.39
T+ ENZ+BLO	49.6±0.17	2287.9±36.2	3845.5±40.4	1.72±0.01 ^{ab}	5.00±2.04
T+ENZ+TO+FO	49.5±0.13	2245.8±30.4	3718.8±78.0	1.69±0.02 ^b	2.50±1.44
T+ENZ+TO+FO+BLO	49.7±0.33	2248.5±41.0	3705.2±48.4	1.69±0.03 ^b	5.00±0.00

^{a,b} Means within the same column with common superscripts do not differ ($p>0.05$)

Table 3. The concentration of *E.coli* in the small intestine segments, log x 10⁶ CFU

Treatments	Duodenum	Jejunum	Ileum
Control	9x10 ⁶ ±1.29 ^{ab}	3x10 ⁶ ±0.91 ^b	4x10 ⁶ ±1.41 ^{ab}
T+ FLV	5x10 ⁶ ±0.91 ^{cd}	4x10 ⁶ ±1.08 ^{ab}	8x10 ⁶ ±1.58 ^a
T+ ENZ	6x10 ⁶ ±1.08 ^{bcd}	7x10 ⁶ ±1.29 ^{ab}	5x10 ⁶ ±1.58 ^{ab}
T+ FLV+ENZ	4x10 ⁶ ±1.22 ^d	4x10 ⁶ ±0.91 ^{ab}	2x10 ⁶ ±0.41 ^b
T+ENZ+TO	11x10 ⁶ ±0.91 ^a	4x10 ⁶ ±1.77 ^{ab}	6x10 ⁶ ±0.91 ^{ab}
T+ENZ+FO	4x10 ⁶ ±0.70 ^d	3x10 ⁶ ±0.91 ^b	8x10 ⁶ ±1.47 ^a
T+ ENZ+BLO	8x10 ⁶ ±1.08 ^{abc}	7x10 ⁶ ±1.58 ^{ab}	5x10 ⁶ ±1.58 ^{ab}
T+ENZ+TO+FO	7x10 ⁶ ±1.08 ^{bcd}	3x10 ⁶ ±1.08 ^b	6x10 ⁶ ±1.08 ^{ab}
T+ENZ+TO+FO+BLO	6x10 ⁶ ±1.29 ^{bcd}	8x10 ⁶ ±2.04 ^a	4x10 ⁶ ±1.08 ^{ab}

^{a,b,c,d} Means within the same column with common superscripts do not differ ($p>0.05$)

CFU Colony Formation Unit

The results regarding the blood parameters are given in Table 4. The erythrocyte levels in the blood of the broilers fed with the diet of triticale-

based and thyme oil, fennel oil, bay leaf oil and flavomycin were found to be significantly low compared to the other groups ($p<0.05$).

Table 4. Effects of treatments on blood parameters

Treatments	Erythrocyte (µL)	Leucocyte (µL)	Hemoglobin (g/dL)	Hematocrit (%)
Control	1.86x10 ⁶ ±0.01x10 ^{6d}	206.05x10 ³ ±1.90 x10 ^{3cd}	11.65±0.28 ^a	42.40±1.43 ^{ab}
T+FLV	2.83 x10 ⁶ ±0.04 x10 ^{6a}	203.60 x10 ³ ±1.61 x10 ^{3d}	11.72±0.52 ^a	42.35±1.32 ^{ab}
T+ENZ	2.34 x10 ⁶ ±0.03 x10 ^{6c}	228.40 x10 ³ ±1.03 x10 ^{3a}	12.35±0.33 ^a	44.22±1.92 ^a
T+ FLV+ENZ	2.36 x10 ⁶ ±0.02 x10 ^{6c}	142.70 x10 ³ ±1.58 x10 ^{3g}	10.35±0.31 ^b	41.75±0.86 ^{ab}
T+ENZ+TO	2.76 x10 ⁶ ±0.03 x10 ^{6a}	211.90 x10 ³ ±2.25 x10 ^{3bc}	10.00±0.50 ^b	38.92±1.06 ^b
T+ENZ+FO	1.84 x10 ⁶ ±0.02 x10 ^{6d}	184.20 x10 ³ ±2.72 x10 ^{3f}	9.67±0.32 ^b	32.92±1.19 ^c
T+ENZ+BLO	2.84 x10 ⁶ ±0.04 x10 ^{6a}	213.02 x10 ³ ±1.50 x10 ^{3b}	9.82±0.36 ^b	39.47±0.36 ^b
T+ENZ+TO+FO	2.59 x10 ⁶ ±0.05 x10 ^{6b}	194.75 x10 ³ ±3.25 x10 ^{3e}	10.15±0.40 ^b	34.82±0.54 ^c
T+ENZ+TO+FO+BLO	1.64 x10 ⁶ ±0.01 x10 ^{6e}	178.32 x10 ³ ±2.42 x10 ^{3f}	9.55±0.44 ^b	33.17±1.84 ^c

^{a,b,c,d,e} Means within the same column with common superscripts do not differ ($p>0.05$)

The group with the diet of plant extracts was found to have the lowest erythrocyte level ($p<0.05$). The group with the diet with enzyme had the highest leucocyte level and the addition of flavomycin decreased the leucocyte level ($p<0.05$). The leucocyte level of the group with the ration of enzyme+thyme oil was similar to the group fed with the diet of enzyme+bay leaf oil and the control group ($p>0.05$). The results were

similar for the group with the diet of enzyme and fennel oil and the group with the thyme, fennel and bay leaf oils ($p>0.05$). The hemoglobin levels were significantly low in the group with the diet of plant extracts and of enzyme and flavomycin. The group with the diet of triticale and enzyme had the highest hematocrit level, the hematocrit level of the group with the diets of fennel oil, and fennel oil, thyme oil and bay leaf oil were found to be

significantly low ($p < 0.05$). The group with the diet of flavomycin with or without enzyme had similar results in terms of hematocrit level compared to the control group ($p > 0.05$).

Discussion

In this study, the additions of plant extracts instead of AGPs were found to have no significant effect on body weight, feed intake and mortality. This finding is similar to the findings of Çabuk et al. (2006) [6] that the addition of the essential oils to the broiler diets did not affect the live weight. However, Al-Ankari et al. (2004) [9] found respectively that the addition of coriander seed to the quail diets, mint and barberry roots to the broiler diets increased the live weight. The experimental treatments did not have any significant effect on the live weight gain. Similar to the findings of this study, Sarica et al. (2005) [10] observed that the addition of AGPs, thyme and garlic powders to wheat-based diet with or without enzymes did not affect the weight gain. However, some researches [9, 11] demonstrated that the use of plant extracts to the broiler diets did not cause any increase in the live weight. Similarly, Amerah et al. (2011) [12] indicated that the addition of essential oils including cinnamaldehyde and thymol to the diets of broilers fed with ground wheat increased the live weight. Cross et al. (2011) [13] reported that the addition of thyme essential oil to the broiler diets decreased the live weight gain.

This study showed no significant differences between the groups in terms of feed intake of the end of trial. However, some researchers [9, 14] found that the addition of plant extracts to the diet increased the feed intake significantly. It is thought that the anti-nutritional materials in the triticale play an important role in decreased feed intake. Moreover, Richter and Lemser (1995) determined that the use of triticale instead of corn in the broiler diets decreased the feed intake [15]. The plant extracts (thyme oil, fennel oil, bay leaf oil) added to the diet of triticale-based diet especially as a combination improved the feed intake significantly. It is seen that similar findings comply with the findings of some researchers [16-18] conducted experiments in this respect. It is thought that the active materials in the essential plant oils that decrease the development of

pathogen microorganisms and ensure better absorption of more nutrients by increasing the digestion enzymes play an important role in the improvement of the feed intake with the addition of plant extracts to the diet. Moreover, Langhout (2000) [19] pointed out that the essential oils improved the production of digestion enzymes and use of intestine content and live weight and feed intake by increasing the liver function; and Jang et al. (2004) [20] indicated that a commercial essential oil combines with lactic acid increased the trypsin and pancreatic amylase activity in broilers. Moreover, it is claimed that the thymol and carvacrol in the thyme can trigger digestion and destroy the pathogen microorganism in the digestion system and may affect the feed intake positively. It is thought that the reason for the significance of the combined use of plant extracts in the groups is the dose and the synergic effect between them. In our study, there was an improvement in feed conversion by adding thyme oil+fennel oil or thyme oil+fennel oil+bay leaf oil with enzyme in triticale-based diet compared to the broiler chickens were fed triticale-based diet only supplemented with enzyme. Their feed conversion was also better than the other groups. Increased digestive capacity and enzyme secretions by adding herbal oils might be the reason for improved feed conversion that were explained before. This study indicated that the dietary treatments did not have any impact on the mortality. Çabuk et al. (2006) [6] claimed that the addition of essential oil to the diets for broilers decreased the mortality.

This study showed that the use of plant extracts in broiler diets did not have any regular positive effects on the number of *E.Coli* in the small intestine segments. This result is in accordance with the findings of Hassan et al. (2004) [14]. These researchers determined that the addition of black cumin seed oil, thyme and quill to the broiler diets did not affect the content of the microorganisms in the intestine. Jang et al. (2004) [20] reported that the addition of plant extracts to the broiler feeds did not affect the number of *E.Coli* in the small intestine but Sarica et al. (2005) [10] demonstrated that thyme and garlic powders in wheat-based diet with or without enzyme decreased the number of *E.Coli* in the small intestine. Jamroz et al. (2005) [21] found that the addition of capsaicin, carvacrol and cinnamaldehyde added to the broiler diets of corn,

wheat and barley decreased the number of *E.Coli* in the small intestine. Moreover, Tiihonen et al. (2010) [11] indicated that the essential oil mixture had positive effects on the cecal microflora for the broilers and increased especially the number of *Lactobacillus* and *E. coli*. Similarly, Suk et al. (2003) [22] reported that the addition of essential oil to the broiler diets decreased the number of *E.coli*.

This study pointed out that the addition of flavomycin, thyme oil and bay leaf oil to the diet increased the erythrocyte level in the blood. However, these extracts had no positive effects on the leucocyte, hemoglobin and hematocrit levels. Some researches [23] reported that the addition of turmeric of 0.5% and 1% to the broiler diets increased the levels of leucocyte and erythrocyte in the blood. Hassan et al. (2004) determined that the addition of black cumin seed, thyme and quill to the broiler diets increased the level of hemoglobin and erythrocytes [14].

Conclusions

It is found out with this study that some plant extracts as an alternative to the AGPs may be easily used in the triticale-soybean meal based broiler diets supplemented with enzyme as an alternative to the corn, which is used as also a human foodstuff and has become an important product for the energy sector in the recent years. However, further researches must conduct to investigate the usage of triticale in poultry diets as alternative to corn.

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