

Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets

S. Sarica^{1#}, A. Ciftci², E. Demir¹, K. Kilinc¹ and Y. Yildirim¹

¹University of Gaziosmanpasa, Faculty of Agriculture, Department of Animal Science, 60240, Tokat, Turkey

²University of Ondokuzmayis, Faculty of Veterinary Medicine, Department of Microbiology, Samsun, Turkey

Abstract

A study was conducted to compare the effects of an antibiotic growth promoter (flavomycin) and two herbal natural feed additives (garlic and thyme) with and without a xylanase-based enzyme complex in wheat-based diets on growth performance, carcass parameters, total plasma cholesterol concentration, intestinal traits and the dry matter content of excreta of broiler chickens. A total of 112 day-old male broiler chicks was randomly assigned to eight groups containing 14 chicks each and raised from 1 to 42 days of age. The control group received the wheat-soyabean meal basal diet. In the treatment groups the basal diet was supplemented with one of the following: an antibiotic, thyme, garlic, an enzyme, the antibiotic plus the enzyme, thyme plus the enzyme or garlic plus the enzyme. During the 42-d growth period there were no significant differences in body weight gain, feed intake and feed conversion ratio of the broilers between dietary treatments. Feeding the diet supplemented with the antibiotic plus the enzyme significantly increased hot and cold carcass yields compared to the diets supplemented with thyme, garlic, enzyme and garlic plus enzyme. Total plasma cholesterol concentration, the dry matter content of excreta and the relative weights of the heart, pancreas, liver, gizzard and spleen were not significantly influenced by dietary treatments. The relative weight of the small intestines of the broilers receiving the diets supplemented with the antibiotic, antibiotic plus enzyme, thyme plus enzyme and garlic plus enzyme were significantly less than those of the broilers fed the basal diet and the diets supplemented with thyme, garlic and enzyme. The basal diet and garlic supplemented diet significantly increased the length of the small intestine compared to the other dietary treatments. Broilers receiving the diet supplemented with antibiotic had significantly lower total aerobic bacterial counts in the small intestines compared to those on the other dietary treatments. The combined supplementation of the antibiotic and enzyme resulted in a significantly lower *E. coli* concentration in the small intestines compared to the basal diet and the other dietary treatments.

Keywords: Broiler, wheat, antibiotic, enzyme, herbs, performance

[#] Corresponding author. E-mail: senaysarica2002@yahoo.com

Introduction

Maize is the major source of energy for poultry diets in many parts of the world. However, maize production in Turkey is insufficient to supply the requirements of the poultry industry, and depends on the use of imported maize. The use of imported maize in feed manufacturing increases the cost of poultry diets. Therefore, alternative cereals such as barley and wheat are important alternatives in poultry diets. Unfortunately, the use of wheat in commercial broiler diets is limited because of its varying content and the presence of soluble non-starch polysaccharides (NSPs) in the endosperm cell wall (Bedford & Classen, 1993). The most important non-starch polysaccharide constituent of wheat is arabinoxylans (Choct & Annison, 1990; Schutte *et al.*, 1993). Several recent studies have indicated that the water-soluble arabinoxylans of wheat had the capacity to bind large amounts of water and increased the viscosity of digesta in the small intestine (Salobir *et al.*, 1995; Knudsen, 2001). Increasing digesta viscosity and water retention inhibits nutrient digestion in the foregut directly by reducing the passage rate and the time that digested nutrients are exposed to the gut wall, and indirectly by stimulating the proliferation of microflora and microbial fermentation (Vukic-Vranjes & Wenk, 1993; Preston *et al.*, 2001). Prolific microflora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids (Engberg *et al.*, 2000; Langhout *et al.*, 2000). As a consequence, this leads to depressed growth performance of broiler chickens and to an increased incidence of disease and management problems associated with sticky droppings and wet litter conditions (Steenfeldt, 1995; Santos Jr

et al., 2004a). These negative effects in poultry had been alleviated by enzyme supplementation. Studies demonstrated that the supplementation of a xylanase-based enzyme complex to wheat-soyabean meal based broiler diets reduced the viscosity of the digesta in the small intestine, increased the growth performance and reduced disease and management problems in poultry (Schutte *et al.*, 1993; Steinfeldt *et al.*, 1998; Santos Jr *et al.*, 2004b). Viscous diets also respond particularly well to the inclusion of antibiotic growth promoters (AGPs) (Elwinger & Teglof, 1991). There is considerable evidence that the anti-nutritive activity of NSP in poultry is related to the gut microflora of the chicken, since supplementation of AGPs to diets increases their nutritive value (Esteve-Garcia *et al.*, 1997). The mode of action of AGPs is mainly related to an inhibiting effect on certain intestinal bacteria that produce toxins or compete with the host for available nutrients. The inhibition of different species of bacteria that may depress dietary fat absorption due to bile acid deconjugation may further explain the working mechanism of AGPs (Feighner & Dashkevicz, 1987). A few studies have been conducted to determine the effects of the combined usage of AGPs and exogenous enzymes in wheat-based broiler diets. Elwinger & Teglof (1991), Vukic-Vranjes & Wenk (1995) and Hock *et al.* (1997) reported that there might be an interaction between exogenous enzymes and AGPs used in broiler diets, and suggested that their effects are additive. This has been confirmed by Schurz *et al.* (1993) Broz *et al.* (1994), Allen *et al.* (1995), Choct *et al.* (1995) and Langhout & Schutte (1995) for broiler diets containing wheat. Antibiotic feed additives have long been used as growth promoters in poultry nutrition. However, concern has been expressed about the potential development of antibiotic resistant bacteria. Consequently, the animal feed industry, exposed to increasing consumer pressure to reduce the use of AGPs in poultry diets, has to find alternative feed additives (Humphrey *et al.*, 2002; Botsoglou *et al.*, 2004). Herbs could be used as alternatives to AGPs in poultry nutrition due to their antimicrobial properties. Many herbs and their bio-active constituents possess a broad antimicrobial activity (Dorman & Deans, 2000; Kamel, 2001; Tucker, 2002; Cross *et al.*, 2003; Lewis *et al.*, 2003). Scientific evidence exists that herbs and plant extracts stimulate the growth of beneficial bacteria and minimize pathogenic bacterial activity in the gastrointestinal tract of poultry (Gill, 1999; Langhout, 2000; Wenk, 2000).

The objective of the present study was to compare the effects of the supplementation of an antibiotic growth promoter and two herbal natural feed additives with and without a xylanase-based enzyme complex to wheat-based broiler diets on the growth performance, carcass parameters, total plasma cholesterol concentration, intestinal traits and the dry matter content of excreta of broilers.

Materials and Methods

A total of 112 day-old male Ross 308 broiler chicks, purchased from a local commercial hatchery, was randomly assigned to eight groups of similar mean weight, comprising 14 chicks each. The chicks were kept in individual wire cages under uniform environmental conditions from 1 to 42 days of age. This study was conducted under the guidelines of the Institutional Animal Care and Use Committee. Temperature was kept at 32 °C for the first week, 28 °C for the second week and 21 °C thereafter. A continuous lighting program was provided during the experiment.

Prior to the formulation of the experimental diet, the feed ingredients were analyzed for their crude protein (N x 6.25), crude fat, crude fibre, starch and total sugar concentrations, according to the methods of the AOAC (1984). Metabolisable energy of feed ingredients was calculated based on equations of Anonymous (1989). All diets were formulated to meet minimum nutrient requirements established by the NRC (1994). The chicks were fed a commercial broiler starter diet (230 g CP/kg and 13.1 MJ ME/kg) from day 1 to 14, a commercial broiler grower diet (210 g CP/kg and 13.4 MJ ME/kg) from day 14 to 35 and a broiler finisher diet (180 g CP/kg and 13.6 MJ ME/kg) from day 35 to 42. The ingredients and the calculated nutrient composition of the basal diet are presented in Table 1.

The experimental diets were designed as:

T1: A wheat-soyabean meal diet (the basal diet)

T2: The basal diet supplemented with an antibiotic growth promoter (1 g Flavomycin/kg)

T3: The basal diet supplemented with Nor-Spice[®] Thyme powder (1 g/kg)

T4: The basal diet supplemented with Nor-Spice S Garlic powder (1g/kg)

T5: The basal diet supplemented with a xylanase-based enzyme complex (1g Yemzim B/kg)

T6: The basal diet supplemented with a xylanase-based enzyme complex and antibiotic growth promoter (inclusion rates as in T2 and T5)

T7: The basal diet supplemented with a xylanase-based enzyme complex and Nor-Spice[®] Thyme powder (inclusion rates as in T3 and T5)

T8: The basal diet supplemented with a xylanase-based enzyme complex and Nor-Spice[®] S Garlic powder (inclusion rates as in T4 and T5)

Table 1 The ingredients and the calculated nutrient composition of the basal diet (as fed basis)

Ingredients	Composition of basal diet (g/kg)		
	Starter (1 to 14 d)	Grower (14 to 35 d)	Finisher (35 to 42 d)
Maize	141.2	180.0	226.2
Wheat	450.0	450.0	450.0
Soyabean meal	287.2	251.0	236.5
Fish meal	45.5	37.8	-
Vegetable oil	42.2	51.3	57.0
Salt	2.5	2.5	2.5
Limestone	12.5	11.5	11.1
Dicalcium phosphate	13.9	12.2	12.8
DL-methionine	1.5	0.2	0.4
Vitamin premix*	2.5	2.5	2.5
Trace mineral premix**	1.0	1.0	1.0
Total	1000	1000	1000
Calculated composition			
Metabolisable energy (MJ/kg)	13.1	13.4	13.6
Dry matter (g/kg)	892	891	890
Crude protein (g/kg)	230	210	180
Crude fibre (g/kg)	25.4	24.9	25.0
Calcium (g/kg)	10.0	9.0	8.0
Available phosphorus (g/kg)	4.5	4.0	3.5
Methionine (g/kg)	5.0	3.8	3.2
Methionine+Cystine (g/kg)	9.3	7.2	6.0
Lysine (g/kg)	13.5	12.0	9.0

*Vitamin premix/kg diet: Vitamin A - 12 000 IU; vitamin D₃ - 1 500 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

**Trace mineral premix/kg diet: Mn - 80 mg; Fe - 60 mg; Zn - 60 mg; Cu - 5 mg; Co - 0.20 mg; I - 1 mg; Se - 0.15 mg

The two herbal feed additives (thyme and garlic) were supplied by the NOR-FEED ApS (Hvidovre, Denmark). Flavomycin used as an AGP was supplied by Kartal Chemistry (Istanbul, Turkey). The enzyme (Yemzim B; Orba Biochemistry Inc. Co., Istanbul, Turkey) contained 300 IU xylanase/g, 20 IU β-glucanase/g, 20 IU hemicellulase/g and 260 IU amylase activity/g as determined by the manufacturer. Enzyme activity was measured using Xylazyme AX Test tablets according to the Xylazyme AX Test Tablet procedure (McCleary 1992; 1995). The substrate was supplied commercially in a ready-to-use tablet form as Xylazyme AX tablets. Xylazyme AX tablets contained Azurine-crosslinked wheat arabinoxylan. According to this procedure, one unit of enzyme activity was the amount of enzyme required to release one micromole of reducing sugar equivalents (as xylose) from arabinoxylan per minute at 40 °C and pH 4.7. The experimental diets in mash form and drinking water were provided *ad libitum*. During the 42 d experimental period, growth performance was evaluated by recording body weight gain, feed intake and feed conversion ratio. Individual body weights of the broiler chicks were recorded at the beginning of the experiment and on a weekly basis thereafter. Feed intakes were recorded weekly. Feed conversion ratio was calculated weekly as the amount of feed consumed per unit of body weight gain. Seven chickens from each group were selected and their excreta were collected for two hours on days 14, 21, 28, 35 and 42 to determine the dry matter

content of excreta. Dry matter analysis of excreta was carried out using standard methods of the AOAC (1984).

At the end of the experiment seven chickens whose body weights were similar to the group average were selected from each group and slaughtered by severing of the branchial vein. This was done to determine the relative weight of internal organs, the hot and cold carcass yield, total plasma cholesterol concentration and the concentration of microorganisms in the small intestine. The weights of the heart, liver, gizzard, spleen, pancreas and empty small intestine were measured individually. The weights of these internal organs were expressed as a percentage of live body weight. The content of the small intestine was emptied and then weighed. The carcasses were immediately plucked, eviscerated, weighed and then chilled overnight in a refrigerator (+4 °C) to measure cold carcass weight. The hot and cold carcass yields were calculated as a percentage of the pre-slaughter live body weights of broiler chickens. Blood samples were collected for determination of total cholesterol concentration in plasma. To prevent coagulation, blood samples were collected in heparinized test tubes and centrifuged at 1,800 x g for 15 min. After centrifugation, plasma was collected and stored at -20 °C for later analysis. Total plasma cholesterol concentrations were determined spectrophotometrically using a commercial kit (Sigma Chemical Co., St. Louis, MO). After slaughtering, the small intestine (from the distal end of the duodenum to the ileocaecal junction) was removed from each bird and put on ice until they were transported to the laboratory for enumeration of microbial populations. One gram of the content was diluted 1:9 (wt/vol) with physiological salt water (log₁₀). Samples were serially diluted from 10⁻¹ to 10⁻⁷. Using these samples total aerobic bacteria was enumerated on nutrient agar plates after incubation at 37 °C from 24 to 48 h and *E. coli* was counted on MacConkey agar (MCA) and eosin methylene blue (EMB) agar incubated at 37 °C from 8 to 12 h (Anonymous, 1992).

The data obtained from the experiment were analyzed, using the SPSSWIN (1994) statistical program with the analysis of variance (ANOVA). Significant differences between treatment means were separated using the Duncan's Multiple Range Test with a 5% probability (Duncan, 1955).

Results and Discussion

The effect of the experimental treatments on body weight gain, feed intake and feed conversion ratio of broiler chickens is presented in Table 2. The chickens receiving the diet containing the garlic had a lower ($P < 0.05$) body weight gain during the first 14 days of the trial than those on the diets supplemented with thyme, enzyme, antibiotic plus enzyme, thyme plus enzyme and garlic plus enzyme. There were no differences ($P > 0.05$) in body weight gains between treatments from days 14 to 35 and from days 1 to 42. These results are in agreement with those of Schutte *et al.* (1993), Aksoy *et al.* (1995), Garcia *et al.* (1999) and Murphy *et al.* (2003) who reported that the supplementation of a xylanase enzyme to wheat-based diets did not have any significant effect on body weight gain of broilers. Likewise, the supplementation of the enzyme with or without the antibiotic did not significantly affect the body weight gains of broilers on wheat-based diets (Allen *et al.*, 1995; Langhout & Schutte, 1995). Vukic-Vranjes & Wenk (1993) reported that neither the supplementation of the antibiotic, avoparcin, nor the supplementation of the antibiotic plus an enzyme complex containing β -glucanase and xylanase had any significant effect on the body weight gain of broilers on a barley-based diet. Choct & Annison (1992) did not record any significant improvement in the performance of broilers by penicillin supplementation of wheat-based diets.

There are limited reports on the performance of broilers receiving wheat-based diets supplemented with garlic or thyme, with or without an enzyme. Cross *et al.* (2002) observed no significant difference in body weight gain between broilers fed a wheat-soyabean meal based diet with or without the thyme herb. Also, the inclusion of thyme oil did not affect body weight gain of broilers over a 42-d growth period (Cross *et al.*, 2003). Contrary to these findings, Allen *et al.* (1995), Esteve-Garcia *et al.* (1997) and Van Campenhout *et al.* (2001) reported that antibiotic supplementation to wheat-based diets significantly improved body weight gain of broilers during the experimental period.

Table 2 The effect on the body weight gain, feed intake and feed conversion ratio of broilers receiving a wheat-soyabean meal based diet supplemented with either an antibiotic growth promoter (flavomycin), herbal natural feed additives (thyme and garlic) with or without a xylanase-based enzyme complex

Performance parameters	Experimental diets								s.e.m.
	T1	T2	T3	T4	T5	T6	T7	T8	
Initial body weight, g	45.41	45.75	45.03	45.03	45.05	45.29	45.33	44.86	0.28
Body weight gain, g									
1 to 14 d	255.4 ^{ab}	260.8 ^{ab}	276.2 ^a	221.2 ^b	265.0 ^a	272.8 ^a	285.4 ^a	289.2 ^a	5.03
14 to 35 d	1472.3	1585.4	1502.1	1389.2	1445.0	1454.6	1559.6	1573.2	21.96
1 to 42 d	2244.2	2357.3	2282.5	2143.7	2237.7	2211.3	2410.1	2384.2	27.48
Feed intake, g									
1 to 14 d	399.2	409.2	418.8	379.6	400.8	414.6	415.0	439.6	4.66
14 to 35 d	2389.6	2473.1	2405.0	2230.0	2346.5	2475.8	2478.5	2542.7	32.44
1 to 42 d	4005.8	4102.3	4026.7	3825.0	3951.2	4069.6	4169.2	4208.2	42.36
Feed conversion ratio, g:g									
1 to 14 d	1.56	1.57	1.52	1.72	1.51	1.52	1.45	1.52	0.22
14 to 35 d	1.62	1.56	1.60	1.61	1.62	1.70	1.59	1.62	0.01
1 to 42 d	1.79	1.74	1.77	1.79	1.77	1.84	1.73	1.77	0.01

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

s.e.m. - standard error of means

T1 - the basal diet (wheat-soyabean meal based);

T2 - the basal diet supplemented with flavomycin;

T3- the basal diet supplemented with thyme;

T4- the basal diet supplemented with garlic;

T5- the basal diet supplemented with a xylanase-based enzyme complex;

T6 - the basal diet supplemented with a xylanase-based enzyme complex and flavomycin;

T7 - the basal diet supplemented with a xylanase-based enzyme complex and thyme;

T8 - the basal diet supplemented with a xylanase-based enzyme complex and garlic

Table 3 The effects of the usage of an antibiotic growth promoter (flavomycin) and two herbal natural feed additives (thyme and garlic) with and without a xylanase enzyme complex in wheat-based broiler diets on the relative weights of some internal organs and carcass yields of the broilers at 42 days of age

Variable	Experimental diets								s.e.m.
	T1	T2	T3	T4	T5	T6	T7	T8	
Hot carcass yield, %	75.08 ^{abc}	76.04 ^{ab}	74.36 ^c	74.94 ^{bc}	74.97 ^{bc}	76.48 ^a	75.50 ^{abc}	74.75 ^{bc}	0.18
Cold carcass yield, %	74.23 ^{abc}	74.72 ^{ab}	73.29 ^{bc}	73.62 ^{bc}	73.57 ^{bc}	75.07 ^a	74.62 ^{abc}	73.24 ^c	0.17
Heart, g (100 g/BW)	0.57	0.58	0.61	0.57	0.60	0.54	0.59	0.58	0.01
Liver, g (100 g/BW)	1.81	1.75	1.85	1.92	1.88	1.73	1.78	1.83	0.02
Gizzard, g (100 g/BW)	1.14	1.15	1.17	1.20	1.20	1.18	1.14	1.13	0.02
Spleen, g (100 g/BW)	0.11	0.12	0.13	0.12	0.13	0.11	0.11	0.12	0.003
Pancreas, g (100 g/BW)	0.20	0.16	0.20	0.18	0.19	0.17	0.16	0.18	0.005
Small intestine, g (100 g/BW)	2.94 ^a	2.46 ^b	2.86 ^a	2.92 ^a	2.81 ^a	2.52 ^b	2.62 ^b	2.54 ^b	0.03
Length of small intestine (cm)	170 ^a	156 ^d	165 ^b	168 ^a	158 ^d	152 ^e	164 ^b	161 ^c	0.83

^{a,b,c} Means within the same row with common superscripts do not differ ($P > 0.05$); s.e.m. - standard error of means; BW – body weight

T1 - the basal diet (wheat-soyabean meal based);

T2 - the basal diet supplemented with flavomycin;

T3 - the basal diet supplemented with thyme;

T4 - the basal diet supplemented with garlic;

T5 - the basal diet supplemented with a xylanase-based enzyme complex;

T6 - the basal diet supplemented with a xylanase-based enzyme complex and flavomycin;

T7 - the basal diet supplemented with a xylanase-based enzyme complex and thyme;

T8 - the basal diet supplemented with a xylanase-based enzyme complex and garlic

Table 4 The effects of the inclusion of an antibiotic growth promoter (flavomycin) and two herbal natural feed additives in broiler diets on total plasma cholesterol concentration, the dry matter content of excreta and the concentrations of the total aerobic bacteria and *E. coli* in the small intestine

Variable	Experimental diets								s.e.m.
	T1	T2	T3	T4	T5	T6	T7	T8	
Total plasma cholesterol, mg/dL	180.6	153.3	169.0	213.7	169.4	245.5	182.7	155.7	13.25
Dry matter content of excreta, %									
14 d	28.74	28.88	31.93	28.18	25.27	26.37	34.15	38.34	1.17
21 d	20.77	19.22	17.28	18.89	19.64	20.13	17.23	17.34	0.49
28 d	21.22	19.61	16.08	20.40	17.52	16.23	20.27	19.21	0.55
35 d	19.52	17.87	18.23	17.69	16.52	17.15	16.20	17.84	0.39
42 d	30.73	28.79	30.21	31.05	29.95	24.05	26.59	29.22	0.82
The concentration of total aerobic bacteria in the small intestine, log x 10 ⁶ CFU	6.80 ^a	6.55 ^e	6.75 ^c	6.78 ^b	6.65 ^d	6.78 ^b	6.74 ^c	6.66 ^d	0.007
The concentration of <i>E. coli</i> in the small intestine, log x 10 ⁶ CFU	6.72 ^a	6.52 ^e	6.67 ^b	6.67 ^b	6.58 ^d	6.43 ^f	6.67 ^b	6.61 ^c	0.007

^{a,b,c,d,e,f} Means within the same row with common superscripts do not differ ($P > 0.05$); s.e.m. - standard error of means; CFU - colony formation unit

T1 - the basal diet (wheat-soyabean meal based);

T2 - the basal diet supplemented with flavomycin;

T3 - the basal diet supplemented with thyme;

T4 - the basal diet supplemented with garlic;

T5 - the basal diet supplemented with a xylanase-based enzyme complex;

T6 - the basal diet supplemented with a xylanase-based enzyme complex and flavomycin;

T7 - the basal diet supplemented with a xylanase-based enzyme complex and thyme;

T8 - the basal diet supplemented with a xylanase-based enzyme complex and garlic

The feed intakes of the birds were not ($P > 0.05$) affected by any of the supplemental treatments, in agreement with the findings of Jeroch *et al.* (1993), Schutte *et al.* (1993) and Jansman *et al.* (1999). Similarly, Langhout & Schutte (1995) reported that the inclusion of avilamycin and a xylanase enzyme preparation separately or combined did not significantly affect the feed intake of broilers on a wheat-based diet, and Engberg *et al.* (2000) and Van Campenhout *et al.* (2001) found no effect on feed intake when an antibiotic was added to a wheat-based broiler diet. No mortalities were recorded over the total feeding period.

As indicated in Table 2, there were no differences ($P > 0.05$) in feed conversion ratios between dietary treatments over the experimental period. These results are consistent with those of Engberg *et al.* (2000) and Van Campenhout *et al.* (2001). They concluded that antibiotic supplementation to the wheat-based broiler diets did not significantly influence feed conversion ratio. Ceylan *et al.* (1998) reported that antibiotic supplementation to rye-based diets had no significant effect on the feed conversion ratio of broilers. Similar results were reported by Garcia *et al.* (1999), Aksoy *et al.* (1995) and Jamroz *et al.* (1995) who tested the effect of a xylanase enzyme preparation and Vukic-Vranjes & Wenk (1993) who compared the supplementation of an antibiotic supplement alone or combined with an enzyme. Contrary to these findings, Esteve-Garcia *et al.* (1997) reported that flavomycin or a xylanase preparation supplement in a wheat-based diet significantly improved the feed conversion ratio of broiler chickens. The observed lack of a growth promoting effect may be associated with the environmental conditions. Well-nourished healthy chicks do not positively respond to growth-promoters when they are housed under clean conditions and at a moderate stocking density. In the present study, the broiler chickens were kept in good hygienic conditions, which would probably result in a decreased efficacy of antibiotics or any dietary herbal additive. The lack of response to enzyme supplementation could be partly explained by the relatively low level of wheat in the broiler diets.

The effects of the supplements on relative weights of internal organs and carcass yields are summarized in Table 3. The relative weights of the heart, liver, gizzard, spleen and pancreas were not ($P > 0.05$) affected by dietary treatments, in agreement with the findings of Hashish *et al.* (1995) who tested the supplementation of the antibiotic, zinc bacitracin, alone or combined with an enzyme complex, kemzyme to barley-based broiler diets. In the present study the feeding of the diet supplemented with the antibiotic plus the enzyme increased ($P < 0.05$) hot and cold carcass yields compared to those receiving the diets containing the thyme, garlic, the enzyme and garlic plus enzyme. A similar observation was reported by Ceylan *et al.* (1998). They concluded that carcass yield was not affected by either enzyme or enzyme plus antibiotic treatments compared to their rye-based control diet. Esteve-Garcia *et al.* (1997) recorded that neither flavomycin nor a xylanase enzyme influenced the carcass yield of birds on a wheat-based broiler diet. Hernandez *et al.* (2004) found no differences in gizzard, liver and pancreas weights of broiler chickens fed wheat-soyabean meal based diets supplemented with an antibiotic and two plant extracts (an essential oil extract from oregano, cinnamon and pepper and a labiatae extract from sage, thyme and rosemary).

The relative weights of the small intestines of broilers fed diets supplemented with an antibiotic, an antibiotic plus an enzyme, thyme plus the enzyme and garlic plus the enzyme were less ($P < 0.05$) than those of broilers fed the basal diet and the diets supplemented with thyme, garlic and the enzyme. This is in agreement with the findings of Yu *et al.* (1998) who found that the increasing replacement of maize by barley increased the relative weight of the small intestine. The β -glucanase supplementation had no significant effect on these traits compared to the basal diet. The basal diet and garlic-supplemented diet increased ($P < 0.05$) the length of small intestine compared to that of the other treatments.

The effect of dietary supplementation of the experimental treatments on total plasma cholesterol concentrations, the dry matter content of excreta and the concentrations of microorganisms in the small intestine is presented in Table 4. No significant differences ($P > 0.05$) were observed in the total plasma cholesterol concentrations between dietary treatments. Unfortunately, little information has been published on the effects of the supplements tested in this study on total plasma cholesterol concentrations. Horton *et al.* (1991) reported that total serum cholesterol concentrations were not significantly affected by the supplementation of dietary garlic powder at different levels (0 and 1 g/kg) over a 35-d growth period. Some studies suggested that commercial garlic oil, garlic powder and commercially available garlic extract may not be hypocholesterolemic (Berthold *et al.*, 1998; McCrindle *et al.*, 1998). However, Chowdhury *et al.* (2002) reported in laying hens on wheat-maize based diet, a linear decrease in total serum cholesterol

concentration with increasing levels (0, 2, 4, 6, 8 or 10%) of garlic. Qureshi *et al.* (1983a) found in broilers on diets containing the equivalent of 1, 2, 4, 6 and 8% garlic paste, that serum cholesterol concentrations reduced by 18, 21, 24 and 25%, respectively. Chowdhury *et al.* (2002) suggested that the relative stability of chemical ingredients in garlic and the duration of the study may effect responses, since Lawson *et al.* (1992) reported that alicin, the potentially active component in garlic, is unstable and poorly absorbed from the digestive tract.

The dry matter content of excreta at days 14, 21, 28, 35 and 42 was not affected ($P > 0.05$) by the dietary treatments. This observation is in agreement with those of Vukic-Vranjes & Wenk (1993), Barrier-Guillot *et al.* (1995) and Salobir *et al.* (1995). Barrier-Guillot *et al.* (1995) and Salobir *et al.* (1995) found that xylanase supplementation did not affect the dry matter content of excreta and Vukic-Vranjes & Wenk (1993) reported that the supplementation of an antibiotic with or without an enzyme complex to barley-based broiler diets did not have a significant effect on the dry matter content of excreta. Replacing the AGP, zinc bacitracin, with rhubarb (*Rheum rhaponticum* WILLD.), as an herb, did not significantly influence the dry matter content of excreta (Gebert *et al.*, 1999).

The concentrations of total aerobic bacteria and *E. coli* in the small intestine were affected ($P < 0.05$) by dietary treatments (Table 4). All dietary treatments decreased ($P < 0.05$) the concentrations of total aerobic bacteria and *E. coli* in the small intestines compared to the non-supplemented basal diet. Broilers fed the diet supplemented with the antibiotic (T2) had lower ($P < 0.05$) total aerobic bacteria counts compared to the basal diet and the other dietary treatments. The combined supplementation of the antibiotic and enzyme (T6) had a lower ($P < 0.05$) *E. coli* concentration compared to the basal diet and the other dietary treatments (Table 4). Results of the present study concur with the results of Jamroz & Kamel (2002) who reported that the dietary herbal treatment resulted in lower *E. coli* counts compared to the control group. Tucker (2002) demonstrated that the supplementation of a mixed herbal product containing garlic, anise, cinnamon, rosemary and thyme to commercial pig diets significantly inhibited the number of *E. coli* in the digestive tract. Hesselman & Aman (1986) reported that viscosity was increased in the presence of water-soluble non-starch polysaccharides, which decreased digesta passage rate and retention time in the lower part of the small intestine (ileum) and in the large intestine (caecum and rectum) of chickens. This resulted in an increase in the microbial population especially the number of bacteria such as *E. coli*, *Clostridium* spp. and *Enterococci*. Contrary to these findings Guo *et al.* (2004) reported that the total aerobic bacteria and *E. coli* counts in the caecum of the birds fed the antibiotic, apramycin, were significantly higher than those of the control group and the groups that received mushroom and herbal extracts.

Conclusions

The results of the present study showed that an antibiotic growth promoter (flavomycin) and two herbal natural feed additives (thyme and garlic) with and without a xylanase-based enzyme complex had no significant effect on the growth performance, total plasma cholesterol concentration, dry matter content of excreta, the relative weights of some internal organs, except for small intestinal traits, hot and cold carcass yields and the concentrations of total aerobic bacteria and *E. coli* in the small intestine when incorporated into wheat-based broiler diets. The broiler chickens in the present study were kept in clean, disinfected conditions of minimal bacterial challenge and in individual wire cages, which would possibly lead to a reduced efficacy of any dietary feed additives. The beneficial effects of these supplements might be observed under less hygienic housing conditions, especially under the separate floor pens equipped with wood shaving litter and when including higher levels of wheat in the diets.

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