Effects of novel feed additives in wheat based diets on performance, carcass and intestinal tract characteristics of quail

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Abstract

The present study was conducted to investigate the effects of the supplementation of an antibiotic growth promoter or novel feed additives with or without a xylanase-based enzyme complex to wheat-based diets on the growth performance, carcass yields and quality and intestinal characteristics (length of the total and small intestine, pH and viscosity of digesta, microflora) of Japanese quail. Eight hundred and forty day-old male quail chicks were randomly assigned to 14 groups of similar mean weight each of which included three replicates of 20 quail. The control group received a wheat-soyabean meal basal diet. In the treatment groups, the basal diet was supplemented with one of the following: an antibiotic, oregano essential oil, cinnamon essential oil, oregano essential oil plus cinnamon essential oil, a probiotic, a mannanoligosaccharide, and the same diets plus an enzyme. During the 35-d growth period there were no significant differences in body weight gain (BWG), feed intake (FI) or feed conversion ratio (FCR) of quail between dietary treatments. The use of oregano essential oil plus cinnamon essential oil or mannanoligosaccharide without the enzyme complex in the quail diets decreased plasma total cholesterol level compared to the control diet. The dietary supplementation of AGP, oregano essential oil, cinnamon essential oil, oregano essential oil plus cinnamon essential oil without the enzyme complex decreased plasma triglyceride levels compared with the control diet. When oregano essential oil, cinnamon essential oil, oregano oil plus cinnamon oil, a probiotic or a mannanoligosaccharide with an enzyme complex were used in a wheat based quail diet, the intestinal viscosity was significantly decreased compared to the control diet. Although the use of essential oils in combination with the enzyme complex, a probiotic and a mannanoligosaccharide with or without the enzyme complex in the wheat based diet significantly reduced the intestinal viscosity compared to the control diet, these treatments negatively decreased plasma total cholesterol and triglyceride.

Keywords: Antibiotic, novel feed additives, performance, carcass yields and quality, intestinal traits
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Introduction

Maize is the major source of energy for poultry diets in many parts of the world. However, poultry producers have considered wheat as alternative to maize because of its competitive price. Unfortunately, wheat in commercial poultry diets is used to a limited extent because of the presence in varied amounts of soluble non-starch polysaccharides (NSPs) in the endosperm cell wall (Mathlouthi *et al*., 2003b). The predominant NSP antinutrients in wheat are arabinoxylans. Several recent studies have shown 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).

Increasing digesta viscosity and water retention inhibit nutrient digestion in the foregut directly by reducing the passage rate of digested nutrients to the gut wall and time of exposure of nutrients to digestive enzymes, and indirectly by stimulating the proliferation of microflora and microbial fermentation (Vukic-Vranjes & Wenk, 1993; Preston *et al*., 2001). As a result, growth performance of broiler chickens was depressed and the incidence of disease and management problems associated with sticky droppings and wet litter conditions were increased (Santos Jr *et al*., 2004a). These negative effects in poultry were reduced by enzyme supplementation to wheat-based diets. Diets that increase the viscosity of digesta in the small intestine also respond particularly well to the inclusion of antibiotic growth promoters (AGPs) (Sarica *et al*.,

2005). The mode of action of AGPs may be explained by an inhibiting effect on certain intestinal bacteria that produce toxins or compete with the host for available nutrients. However, there are some studies in which the effects of the combined use of AGPs and exogenous enzymes in wheat based broiler diets have been investigated (Allen *et al.*, 1995; Vukic-Vranjes & Wenk, 1995; Esteve-Garcia *et al*., 1997). Antibiotics have been used by the poultry industry and poultry veterinarians for a specific period of time, at first primarily to control disease and more recently to enhance growth and improve feed efficiency (Waldroup *et al*., 2003a). However, the AGPs have been under scrutiny for many years and have been removed from the market in many countries due to increased public concern over the development and spread of antibiotic resistance in bacteria and the possible presence of antibiotic residuals in poultry products. Consequently, feed manufacturers and the animal growers have to find novel feed additives that may be used to reduce the problems related to the withdrawal of AGPs from diets and enteric disease in poultry (Fritts & Waldroup, 2003; Ayed *et al*., 2004).

Probiotics, prebiotics or plant extracts could be used as alternatives to AGPs in poultry nutrition due to their effects on microflora. Probiotics are defined as viable microbial feed additives which assist in the establishment of an intestinal population which is beneficial to the animal and antagonistic to pathogen microorganisms (Green & Sainsbury, 2001; Shane, 2001).

Mannanoligosaccharides (MOS) derived from mannan yeast cell surfaces act as high affinity ligands offering a competitive binding site for the pathogen microorganisms (Parks *et al*., 2001). Consequently, pathogens are unable to bind to the intestinal mucosa (McCann *et al.*, 2006). Pathogens with the mannose specific fimbriae absorb to the MOS instead of attaching to intestinal epithelial cells and consequently move through the intestine without colonization (Fritts & Waldroup, 2003). The supplementation of MOS to diets increases the lactic acid content and the proliferation of beneficial species of bacteria in the gut and boosting the immune system (McCann *et al*., 2006).

Recently, herbs, spices and various plant extracts have received increased attention as possible AGP replacements (Hernandez *et al*., 2004). Various plant extracts and their bioactive components possess broad antimicrobial activities (Cross *et al*., 2003; Lewis *et al*., 2003). The phenolic compounds such as carvacrol and thymol present in the essential oil from oregano exhibit considerable antimicrobial and antifungal activity (Basilico & Basilico, 1999). Thymol and carvacrol disrupt the membrane integrity, which affects pH homeostasis and equilibrium of inorganic ions (Lambert *et al*., 2001). The antimicrobial properties of the cinnamon essential oil are mainly related to its cinnamaldehyde content followed by eugenol and carvacrol content (Tabak *et al.*, 1999). They have antibacterial activity due to the preventive effect by the cinnamon essential oil and its constituents on the pathogen microorganisms (Hernandez *et al*., 2004). Scientific evidence exists that herbs and plant extracts stimulate the growth of beneficial bacteria and limit numerous pathogenic bacterial activities in the gut of poultry (Wenk, 2000).

The objective of the present study was to compare the effects of the supplementation of an AGP or novel feed additives with or without a xylanase-based enzyme complex to wheat-based diets on the growth performance, carcass yields and quality, plasma total cholesterol and triglyceride level, and intestinal tract characteristics of quail.

Materials and Methods

Eight hundred and forty day-old male Japanese quail chicks (*Coturnix coturnix japonica*) obtained from a commercial hatchery (Ozak, Tokat, Turkey) were wing-banded, weighed and randomly assigned to 14 groups of similar mean weight, each of which included three replicates of 20 chicks. The chicks were kept in wire cages equipped with nipple drinkers (Cimuka, Ankara, Turkey) under standard environmental conditions from hatch until 5 wk 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 programme was provided during the experiment. Prior to experimental diet formulation, feed ingredients were analyzed for crude protein, crude fat, starch and total sugar content, according to the methods of the Association of Analytical Chemists (AOAC, 2000). Metabolizable energy of the feed ingredients was calculated based on analyzed values of feedstuffs (WPSA, 1989). All diets were formulated to meet minimum nutrient requirements established by the National Research Council (NRC, 1994). The experimental diets in mash form and drinking water were provided *ad libitum*. The ingredients and the calculated nutrient composition of the basal diet are presented in Table 1.

The experimental diets were as follows:

T1: A wheat-soyabean meal diet (the basal diet)
T2: The basal diet plus an AGP (1 g flavomycin/kg)
T3: The basal diet plus a xylanase-based enzyme complex (1 g Yemzim B/kg)
T4: The basal diet plus oregano essential oil (1 g *Origanum onites* L./kg)
T5: The basal diet plus cinnamon essential oil (1 g *Cinnamomum verum* J./kg)
T6: The basal diet plus oregano essential oil (500 mg *Origanum onites* L./kg) plus cinnamon essential oil (500 mg *Cinnamomum verum* J./kg)
T7: The basal diet plus a probiotic (1 g Protexin®/kg)
T8: The basal diet plus a mannanoligosaccharide (1 g Bio-Mos/kg)
T9: T2 plus a xylanase-based enzyme complex (inclusion rates as in T2 and T3)
T10: T4 plus a xylanase-based enzyme complex (1 g Yemzim B/kg)
T11: T5 plus a xylanase-based enzyme complex (1 g Yemzim B/kg)
T12: T6 plus a xylanase-based enzyme complex (1 g Yemzim B/kg)
T13: T7 plus a xylanase-based enzyme complex (1 g Yemzim B/kg)
T14: T8 plus a xylanase-based enzyme complex (1 g Yemzim B/kg)

Oregano essential oil (*Origanum onites* L.) was provided by Terme Agricultural Products Ltd. (Izmir, Turkey). Cinnamon essential oil (*Cinnamomum verum* J.) was supplied by Laure Beneficial Herbs Ltd. (Antalya, Turkey). Flavomycin used as an AGP was supplied by Kartal Chemistry (Istanbul, Turkey). Protexin used as a probiotic was provided by Novartis (Istanbul, Turkey). Bio-Mos used as a mannanoligosaccharide was supplied by Ares Food, Agriculture and Animal Industry and Trade Ltd. Co. (Izmir, Turkey). Bio-Mos contained 30% mannan, 30% glucan and 12.5% protein. Protexin included 1.89×10^{10} *Lactobacillus plantarum*, 3.09×10^{10} *Lactobacillus delbrueckrii* subsp. *bulgaricus*, 3.09×10^{10} *Lactobacillus acidophilus*, 3.09×10^{10} *Lactobacillus rhamnosus*, 3.00×10^{10} *Bifidobacterium bifidum*, 6.25×10^{10} *Streptococcus salivarius* subsp. *thermophilus*, 8.85×10^{10} *Enterococcus faecium*, 7.98×10^{10} *Aspergillus oryza* and 7.98×10^{10} *Candida pintolopesii* as a cfu/kg.

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-cross-linked 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.

During the 35-d experimental period, the growth performance of quail was evaluated by recording body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). Individual BW of quail was recorded at the beginning of the experiment and on a weekly basis thereafter. The FCR was calculated weekly as the amount of feed consumed per unit of BWG. At the end of the experiment, six quails whose body weights were similar to the group average were selected from each of the replicate groups in each treatment and slaughtered by severing the jugular vein, to determine some measurements of carcass yield and quality. These measurements included hot and cold carcass yields and intestinal tract characteristics. The carcasses were immediately plucked, processed (removal of head and feed), eviscerated (removal of gastrointestinal tract), weighed and then chilled overnight in a refrigerator (+4 °C) in order to scale for cold carcass weight. The hot and cold carcass yields were calculated as a percentage of the pre-slaughter BW of the quail. Blood samples were collected for determination of triglyceride and total cholesterol in plasma. The aim was to investigate the effects of the supplemental feed additives in wheat based diets on the fat digestibility in the small intestine. To prevent coagulation, blood samples were collected in heparinized test tubes and centrifuged at 1800 x g for 15 min. After centrifugation, plasma was collected and stored at –20 °C for later analysis. Plasma biochemistry parameters were determined spectrophotometrically using commercial kits (Audit Autoanalyser Test Kits, Ireland). At the end of the experiment, three quails, whose body weights were similar to the group average, were selected from each of the replicate groups of each treatment and slaughtered for enumeration of microbial populations.

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

Ingredients	g/kg diet
	Basal diet
Wheat (hard winter)	600
Soyabean meal	332
Fish meal	10.4
Vegetable oil	22.6
Dicalcium phosphate	13.5
Limestone	11.0
Salt	2.80
Vitamin premix*	2.50
Trace mineral premix**	1.00
DL-methionine	2.18
L-Lysine	1.89
Total	1000
Calculated composition	
Dry matter	891
Metabolizable energy (MJ/kg)	12.16
Crude protein	240
Crude fat	39.9
Crude fibre	31.4
Crude ash	61.2
Calcium	9.0
Total phosphorus	7.5
Available phosphorus	4.3
Methionine	5.5
Methionine+Cystine	9.3
Lysine	13.3
Trytophan	2.8
Arginine	14.8

*Vitamin premix/kg diet: 12 000 IU vitamin A; 1 500 IU vitamin D_3; 50 mg vitamin E; 5 mg vitamin K_3; 3 mg vitamin B_1; 6 mg vitamin B_2; 5 mg vitamin B_6; 0.03 mg vitamin B_{12}; 25 mg niacin; 12 mg Ca-D-pantothenate; 1 mg folic acid; 0.05 mg D-biotin; 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride.
**Trace mineral premix/kg diet: 80 mg Mn; 60 mg Fe; 60 mg Zn; 5 mg Cu; 0.20 mg Co; 1 mg I; 0.15 mg Se.

After slaughtering, the small intestine (from the distal end of the duodenum to the ileocaecal junction) was removed from each quail 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 (log_{10}). 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).

Intestinal viscosity was determined in a separate group of quail with the same diets as for the performance study. The 35-d quail used for the digesta viscosity measurements were fasted overnight (8 h) and then given *ad libitum* access to feed for 3 h before sampling. Six other quails from each group were then weighed and euthanased with carbon dioxide. Digesta samples included the contents from Meckel's diverticulum to the ileo-caecel junction. Approximately 2.0 g of digesta was placed into micro-centrifuge tubes and centrifuged at 10.000 x g for 5 min. Then, 0.5 mL of supernatant was measured in a Brookfield Digital Viscometer (Model LVDV-II, Brookfield Engineering Laboratories, Inc., Stoughton, MA) at 40 °C and 50 rpm.

The data obtained from the experiment were analyzed using SPSSWIN (1994) statistical programmes with the ANOVA. Significant differences among treatment means were separated using Duncan's multiple range test with a 5% probability (Duncan, 1955).

Results and Discussion

The effects of the experimental treatments on BWG, FI and FCR of the quail are presented in Table 2. As shown in Table 2, there were no differences (P >0.05) in BWG between dietary treatments from days 0 to 35. These results are in agreement with those of Allen *et al.* (1995) and Langhout & Schutte (1995) who reported that the body weight gains of broiler chickens fed wheat-based diets were not significantly affected by the supplementation of the enzyme with or without the AGP. The supplementation of a xylanase enzyme to a wheat-based diet did not have any significant effect on body weight gain of broilers (Garcia *et al*., 1999; Murphy *et al*., 2003). Choct & Annison (1992) did not find any significant improvement in the performance of broilers by supplementing diets containing wheat with penicillin. Vukic-Vranjes & Wenk (1993) recorded 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 BWG of broilers fed a barley-based diet. Published reports on the use of mannanoligosaccharide in broiler diets are sparse and show inconsistent response. This situation may be due to difference in its level of incorporation to diets. Eren *et al.* (1999) fed broiler chicks diets with 1 g/kg of Bio-Mos to 35 d and reported no significant differences in BWG compared to a negative control. Shafey *et al.* (2001) and Garces-Narro *et al.* (2006) pointed out that the supplementation of Bio-Mos to broiler diets did not influence the BWG. Ceylan *et al.* (2003) and Gunal *et al.* (2006) reported that the dietary supplementation of a probiotic did not have any effect on BWG. In addition, the supplementation of a probiotic to barley-based broiler diets with an enzyme with β-glucanase activity had no beneficial effects on BWG (Torki, 2006). However, the results on the beneficial effects of probiotics on BWG were reported by several researchers (Cavazzoni *et al.*, 1998; Kalavathy *et al.*, 2003; Gracia *et al.*, 2006). Likewise, Allen *et al.* (1995), Esteve-Garcia *et al.* (1997) and Van Compenhout *et al.* (2001) indicated that the BWG of broilers during the experimental period was significantly improved by the antibiotic supplementation.

There are limited studies on the performance of broiler chickens fed wheat-based diets supplemented with essential oil, with or without an enzyme. Cross *et al.* (2003) reported that the inclusion of thyme oil did not influence BWG of broilers over a 42-d growth period. Lee *et al.* (2004b) reported that the BWG of broilers fed rye-based diets supplemented with thymol, cinnamaldehyde or a commercial blend of essential oil components (CRINA®Poultry) was not significantly affected compared with the corn-based control diet.

As indicated in Table 2, there were no differences (P >0.05) in FI between dietary treatments over the experimental period. Similar results were reported by Engberg *et al.* (2000) and Van Compenhout *et al.* (2001) who found no significant effect on FI when an antibiotic was added to a wheat-based broiler diet. Langhout & Schutte (1995) also reported that the FI of broilers fed a wheat-based diet was not significantly affected by the inclusion of a xylanase enzyme preparation separately or combined. These results are consistent with those of Sarica *et al.* (2005) and Lee *et al.* (2004b). Sarica *et al.* (2005) indicated that the FI of broilers receiving diets containing an AGP (flavomycin) or two herbal natural feed additives (garlic and thyme) with and without a xylanase-based enzyme complex in wheat-based diets was not significantly affected. Lee *et al.* (2003b; 2004b) observed no significant difference in FI between broilers fed a rye-soyabean based diet with or without thymol, cinnamaldehyde or CRINA® Poultry and maize-soyabean based control diet. Gunal *et al.* (2006) pointed out that the FI of broiler chickens was not affected by the supplementation of a probiotic (0.1% protexin) to diet. Contrary to these findings, Torki (2006) reported that the supplementation of a probiotic to a barley-based diet significantly decreased the FI of broiler chickens compared to the control diet. Spais *et al.* (2003) showed that feeding a diet supplemented with prebiotic significantly increased feed intake compared to the control diet.

The FCR of quail was not (P >0.05) affected by any of the supplemental treatments, in agreement with the findings of Engberg *et al.* (2000) and Van Compenhout *et al.* (2001). They concluded that FCR was not significantly influenced by the antibiotic supplementation to the wheat-based broiler diets. Similar findings were reported by Garcia *et al.* (1999) and Jamroz *et al.* (1995) who investigated the effect of a xylanase enzyme complex and Vukic-Vranjes & Wenk (1993) who compared the supplementation of an antibiotic supplement alone or combined with an enzyme complex. Esteve-Garcia *et al.* (1997) reported that the FCR of broiler chickens was significantly improved by flavomycin and a xylanase enzyme supplement in a wheat-based diet. Lee *et al.* (2003b) pointed out that FCR of female broilers was not significantly different among diets containing thymol, cinnamaldehyde and a commercial preparation of essential oil components (CRINA®Poultry). Likewise, Lee *et al.* (2004b) pointed out that the FCR of broiler chickens was not

significantly different between diets containing thymol, cinnamaldehyde or CRINA® Poultry and a maize-based diet. Esteve-Garcia *et al.* (1997) reported that the FCR of broiler chickens was significantly improved by flavomycin and a xylanase enzyme supplement in a wheat-based diet. The observed lack of a growth promoting effect may be associated with the variability in the levels of the novel feed additives used in the diet and the environmental conditions. Well-nourished healthy chicks may not positively respond to growth promoting supplements when they are housed under clean conditions and at a moderate stocking density (Botsoglou *et al*., 2004).

The effects of the dietary treatments on plasma total cholesterol and triglyceride levels and carcass yields are summarized in Table 3. There were differences for plasma total cholesterol and triglyceride levels among dietary treatments ($P < 0.05$). The dietary supplementation of oregano essential oil, cinnamon essential oil, oregano essential oil plus cinnamon essential oil, probiotic or mannanoligosaccharide without an enzyme complex had the same effect on plasma total cholesterol level compared to the diet supplemented with an AGP. The use of oregano essential oil, cinnamon essential oil or a probiotic in the quail diet without the enzyme complex did not ($P > 0.05$) affect plasma total cholesterol level compared to the control diet, but where mannanoligosaccharide replaced the probiotic this decreased ($P < 0.05$) plasma total cholesterol level compared to the control diet. The use of the enzyme complex alone or combined with AGP or novel feed additives increased plasma cholesterol level compared to the control diet. The dietary supplementation of AGP, oregano essential oil, cinnamon essential oil, oregano essential oil plus cinnamon essential oil without the enzyme complex decreased ($P < 0.05$) plasma triglyceride level compared with the control diet. The dietary supplementation of the enzyme complex alone or combined with oregano essential oil, cinnamon essential oil, oregano essential oil plus cinnamon essential oil, probiotic or mannanoligosaccharide increased the plasma triglyceride level compared to the control diet.

Table 2 The effects of feeding a wheat-soyabean meal based diet supplemented with either an antibiotic growth promoter or novel feed additives with or without a xylanase-based enzyme complex on body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of quail from 0 to 35 d

Treatments	Performance parameters			
	Initial BW, g (± s.e.)	BWG, g (± s.e.)	FI, g (± s.e.)	FCR, g/g (± s.e.)
Control	7.71 ± 0.30	165 ± 1.8	548 ± 10.1	3.32 ± 0.025
Antibiotic	7.69 ± 0.28	165 ± 4.4	563 ± 20.2	3.42 ± 0.078
Enzyme	7.68 ± 0.28	162 ± 1.6	543 ± 2.8	3.36 ± 0.040
Oregano	7.71 ± 0.24	161 ± 1.1	535 ± 7.6	3.33 ± 0.047
Cinnamon	7.69 ± 0.28	163 ± 1.4	553 ± 15.7	3.40 ± 0.102
Oregano+Cinnamon	7.65 ± 0.27	162 ± 2.5	546 ± 11.1	3.36 ± 0.016
Probiotic	7.65 ± 0.27	159 ± 5.1	534 ± 30.0	3.36 ± 0.091
Prebiotic	7.67 ± 0.28	159 ± 1.5	547 ± 11.4	3.45 ± 0.078
Antibiotic+Enzyme	7.69 ± 0.28	164 ± 3.5	555 ± 14.1	3.39 ± 0.038
Oregano+Enzyme	7.65 ± 0.26	161 ± 1.7	551 ± 18.1	3.43 ± 0.077
Cinnamon+Enzyme	7.64 ± 0.26	166 ± 3.5	542 ± 11.6	3.26 ± 0.006
Oregano+Cinnamon.+Enzyme	7.66 ± 0.27	166 ± 3.1	573 ± 2.9	3.46 ± 0.049
Probiotic.+Enzyme	7.68 ± 0.30	164 ± 3.9	553 ± 2.2	3.37 ± 0.090
Prebiotic+Enzyme	7.64 ± 0.28	166 ± 4.5	556 ± 2.8	3.36 ± 0.099
P value	NS	NS	NS	NS

s.e. - standard error.
NS - not significant; $P > 0.05$.

It is accepted that the antinutritional effect of wheat is mediated by its NSP constituents that raise the viscosity of gut contents and alter the microflora. A decrease in intestinal viscosity associated with reduced bacterial fermentation by enzyme supplementation may increase fat digestion. In the present study, increased fat digestion may have enhanced plasma cholesterol and triglyceride levels. There is evidence that the high lipid digestibility seen in broiler chickens fed wheat-based diet may be due to bacterial reduction in the small intestine and subsequent decreased deconjugation of bile acids by enzyme supplementation (Lee *et al*., 2004b).

Unfortunately, little information has been published on the effects of the dietary supplements studied in this research on the plasma cholesterol and triglyceride levels. Various components of essential oils may exhibit a hypocholesterolaemic effect in chickens (Yu *et al*., 1994; Elson, 1995). The cholesterol-lowering effect of essential oil constituents has been ascribed to inhibition of 3-hydroxy-3-methylglutaryl coenyzme A reductase, which is the rate limiting step in cholesterol synthesis (Lee *et al.*, 2003a; b). It was determined that there are no significant differences among essential oils in terms of plasma total cholesterol and triglyceride levels. The results related to the plasma total cholesterol level of quail in the present study are consistent with those of Lee *et al.* (2004c). Lee *et al.* (2004c) reported that no significant effect on the plasma cholesterol level of female broiler chickens was observed when carvacrol, cinnamaldehyde or a commercial essential oils preparation were supplemented to the diet containing carboxymethyl cellulose. The plasma cholesterol level in female broiler chickens was not significantly influenced by the supplementation of thymol, cinnamaldehyde or a commercial essential oil preparation to the diet containing carboxymethyl cellulose, which is a non-fermentable, viscous fibre (Lee *et al.*, 2003a; 2004a). Case *et al.* (1995) reported that dietary carvacrol and thymol did not show any hypocholesterolaemic effects. Contrary to these findings, Lee *et al.* (2004a) observed that the supplementation of cinnamaldehyde significantly increased plasma total cholesterol level compared to those of female broiler chickens fed the diets supplemented with thymol or CRINA® Poultry that contains 29% of active components, including thymol, but not cinnamaldehyde or rye-based diet without any supplements. However, it is known that the absence or presence of cholesterolaemic effects of essential oils in an animal depend on breed, gender, age and the composition of the feed (Lee *et al.*, 2003b).

In the present study, plasma total cholesterol level was not affected by the supplementation of a probiotic compared to the wheat-based control diets without any feed additives ($P > 0.05$). Santoso *et al.* (1995) found that supplementaton of *B. subtilis* to broiler chickens' diets did not significantly decrease the serum total cholesterol. However, recently, Kalavathy *et al.* (2003) reported that the supplementation of Lactobacillus cultures reduced serum total cholesterol and triglycerides in broilers from 21 to 42 d of age.

There are different hypotheses about the mode of action of probiotics on the plasma cholesterol level. Gilliland *et al.* (1985) hypothesised that lactic acid bacterial strains are able to incorporate cholesterol into the cellular membrane of the organism, thus, less cholesterol will be absorbed into blood. The hypocholesterolaemic effect may result in the ability of lactic acid bacteria to produce bile salt hydrolase to the enzyme responsible for bile salt deconjugation in the intestine. Another reason for the reduction of cholesterol in probiotic-fed hosts is that probiotics may inhibit hydroxymethyl-glutaryl-coenzyme A, an enzyme which is involved in the cholesterol-synthesising pathway (Fukushima & Nakano, 1995).

The mechanism involved in the overall hypocholesterolaemic effect of MOS supplementation is not fully documented. However, MOS as a prebiotic is considered as substrate for lactic acid producing bacteria such as *Lactobacillus* spp. and *Bifidobacterium bifidum* (Van Loo, 2004). It is reported that some *Lactobacillus* spp. are able to incorporate cholesterol into the cellular membrane of the organism, so cholesterol assimilation by *Lactobacillus* in turn decreases cholesterol absorption in the system (Kannan *et al.*, 2005).

It has been pointed out that dietary thymol, carvacrol or cinnamaldehyde decreased serum cholesterol concentrations in chickens. The hypocholesterolemic effect of these feed additives has been ascribed to inhibition of 3-hydroxy-3-methylglutaryl coenyzme A reductase in the rate-controlling enzyme of the cholesterol synthetic pathway (Lee *et al.*, 2003a).

As shown in Table 3, there were no differences ($P > 0.05$) in hot and cold carcass yields of 35-d-old quail between dietary treatments. A similar observation was reported by Esteve-Garcia *et al.* (1997) who recorded that neither flavomycin nor a xylanase enzyme influenced the carcass yield of quail on a wheat-based broiler diet. Ceylan *et al.* (1998) concluded that carcass yield was not affected by either enzyme or

enzyme plus antibiotic treatments compared to their rye-based control diet. Waldroup *et al.* (2003a; b) demonstrated that antibiotic or prebiotic supplementation did not significantly affect carcass yield of broiler chickens. The effects of dietary treatments on the length of total and small intestine and pH value of the small intestine are given in Table 4. No significant differences were observed for these parameters among dietary treatments.

Table 3 The effects of dietary treatments on plasma some parameters, carcass yields and quality[1]

Treatments	Variable				
	Total cholesterol (g/dL) (± s.e.)	Triglyceride (g/dL) (± s.e.)	Preslaughter body weight (g) (± s.e.)	Hot carcass yield (%) (± s.e.)	Cold carcass yield (%) (± s.e.)
Control	170^{d} ± 4.34	60^{d} ± 1.37	182 ± 8.29	56 ± 1.77	56 ± 1.88
Antibiotic	158de ± 3.91	39^{g} ± 7.50	186 ± 6.62	58 ± 2.34	57 ± 2.17
Enzyme	219^{ab} ± 4.06	106^{ab} ± 5.17	184 ± 8.34	57 ± 1.41	56 ± 1.39
Oregano	156de ± 2.84	49^{efg} ± 2.63	193 ± 6.17	56 ± 2.33	55 ± 2.33
Cinnamon	160de ± 3.90	41^{g} ± 2.02	184 ± 10.06	56 ± 2.41	55 ± 2.32
Oregano+Cinnamon	150^{e} ± 1.10	44^{fg} ± 1.97	180 ± 9.30	58 ± 2.59	58 ± 2.61
Probiotic	159de ± 3.77	54^{def} ± 1.76	179 ± 7.56	57 ± 1.44	57 ± 1.53
Prebiotic	152^{e} ± 2.50	57de ± 6.11	187 ± 9.45	57 ± 1.82	56 ± 1.79
Antibiotic+Enzyme	200^{c} ± 8.99	80^{c} ± 2.33	191 ± 9.00	57 ± 2.33	56 ± 2.46
Oregano+Enzyme	207^{bc} ± 4.63	84^{c} ± 9.61	180 ± 11.69	59 ± 2.39	59 ± 2.43
Cinnamon+Enzyme	210^{abc} ± 10.81	57de ± 2.93	184 ± 7.49	56 ± 2.17	56 ± 2.05
Oregano+Cinnamon+Enzyme	195^{c} ± 4.10	96^{b} ± 1.15	187 ± 8.78	56 ± 1.81	55 ± 1.69
Probiotic+Enzyme	205^{bc} ± 7.22	109^{a} ± 1.76	180 ± 8.75	57 ± 1.86	56 ± 1.77
Prebiotic+Enzyme	225^{a} ± 4.06	116^{a} ± 4.33	186 ± 6.47	56 ± 2.29	56 ± 2.23

[1] Values represent the average of three quail in each group at 35-d of age
[a-g] Column means within the common supercripts do not differ (P >0.05); s.e. - standard error.

The effects of dietary supplements on the intestinal viscosity in quail are summarized in Table 5. Quail receiving the control diet, diet supplemented with oregano essential oil or oregano essential oil plus cinnamon essential oil had the highest viscosity in the intestine compared with other dietary treatments (P <0.05). The supplementation of cinnamon essential oil to the basal diet without the enzyme supplementation decreased the intestinal viscosity compared with the diet supplemented with oregano essential oil or oregano essential oil plus cinnamon essential oil without the enzyme complex (P <0.05). When oregano essential oil, cinnamon essential oil or oregano oil plus cinnamon oil with an enzyme complex, an antibiotic, a probiotic or a mannanoligosaccharide with or without an enzyme complex were used in a wheat based quail diet, the intestinal viscosity was positively decreased compared to the control diet (P <0.05). These novel feed additives may have positively affected the viscosity in the intestine due to their positive effect on the intestinal microflora. Bacterial β-galactosidase is mainly produced by bifidobacteria and lactobacilli (Lay *et al.*, 2004). Bacterial glycolytic enzymes play an important role in the fermentation of undigested carbohydrates. β-galactosidase contributes to the hydrolysis of glucose monomers from nonstarch polysaccharides (e.g., cellulose, β-glucans) (Pool-Zoobel *et al.*, 2002). Increasing viscosity in the intestine inhibits the absorption of nutrients by decreasing the gastrointestinal passage rate. Enzyme supplementation to diet inhibits this negative effect and increases the absorption of digesta in the foregut. As

a result, the supplementation of enzymes that decrease viscosity results in improving the digestion and absorption process by increasing the gastrointestinal passage rate and increasing the diffusion of digestive enzymes and the secretion of endogenous enzymes (Van der Klis *et al.*, 1993). Quail fed a diet supplemented with antibiotic or novel feed additives with the enzyme complex had a lower intestinal viscosity compared with the control diet (P <0.05).

Table 4 The effects (± s.e.) of dietary treatments on intestinal tract characteristics[1]

Dietary treatments	Variable		
	Total intestine length Cm	Small intestine length cm	Small intestine pH
Control	68 ± 0.40	62 ± 0.72	6.51 ± 0.04
Antibiotic	65 ± 1.85	59 ± 1.61	6.36 ± 0.03
Enzyme	62 ± 0.29	57 ± 0.30	6.41 ± 0.10
Oregano	63 ± 1.56	58 ± 1.29	6.53 ± 0.05
Cinnamon	62 ± 0.86	54 ± 1.89	6.52 ± 0.11
Oregano+Cinnamon	63 ± 1.77	57 ± 1.43	6.23 ± 0.09
Probiotic	64 ± 1.26	59 ± 1.45	6.52 ± 0.08
Prebiotic	65 ± 1.21	58 ± 2.75	6.23 ± 0.07
Antibiotic+Enzyme	64 ± 2.12	59 ± 1.82	6.52 ± 0.04
Oregano+Enzyme	63 ± 2.84	58 ± 2.68	6.51 ± 0.12
Cinnamon+Enzyme	62 ± 0.42	57 ± 0.26	6.40 ± 0.07
Oregano+Cinnamon+Enzyme	62 ± 2.82	58 ± 2.46	6.55 ± 0.02
Probiotic.+Enzyme	65 ± 2.95	59 ± 2.72	6.44 ± 0.10
Prebiotic+Enzyme	68 ± 1.49	62 ± 1.37	6.39 ± 0.11
P value	NS	NS	NS

[1] Values represent the average of three quail in each group at 35-d of age; s.e. - standard error.
NS - not significant; P >0.05.

Likewise, Lee *et al.* (2004b) indicated that the viscosity of jejunal and ileal digesta was not significantly influenced by the supplementation of essential oil components compared to a rye based control diet without any feed supplements. Esteve-Garcia *et al.* (1997) reported that the viscosity of the intestinal content in the wheat based broiler diet was decreased by the xylanase preparation and flavomycin supplementation in combination.

The concentrations of the total aerobic bacteria and *E. coli* in the small intestine were not affected by the dietary treatments (P >0.05) (Table 5). These results are in agreement with the findings of Jin *et al.* (1998) who reported that there were no significant differences in coliform or total aerobic bacteria counts in the small intestine of broilers fed diets with or without Lactobacillus supplementation during the whole experimental period.

Gut microflora and performance parameters were unaffected by any of the feed additives used here in healthy Japanese quail kept in clean, disinfected conditions of minimal bacterial challenge, in wire cages and at a moderate stocking density.

Conclusions

The results of the present study indicated that an AGP or novel feed additives with or without a xylanase-based enzyme complex had no significant effect on the growth performance, preslaughter body weight, hot and cold carcass yields, length of the total and small intestine, pH value of the small intestine, total aerobic bacteria or *E. coli* concentrations of the small intestine.

The supplementation of oregano essential oil plus cinnamon essential oil without the enzyme to wheat based quail diets decreased plasma total cholesterol and triglyceride levels. MOS supplementation without the enzyme decreases plasma total cholesterol; however an increase of total cholesterol and triglyceride levels was observed with the use of the enzyme complex alone or combined with AGP or novel feed additives compared to the control diet.

Table 5 The effects of dietary treatments on the viscosity of digesta, total aerobic bacteria and *E. coli* concentration of small intestine in quail[1]

Dietary treatments	Variable		
	Viscosity, mPa.s. (± s.e.)	Total aerobic bacteria, logx10^6 CFU (± s.e.)	*E. coli*, logx10^6 CFU (± s.e.)
Control	3.66^{bc} ± 0.19	5.83 ± 0.36	6.00 ± 0.23
Antibiotic	2.66^{d} ± 0.17	5.08 ± 0.28	5.40 ± 0.19
Enzyme	2.25^{def} ± 0.12	5.28 ± 0.24	5.59 ± 0.12
Oregano	4.39^{ab} ± 0.21	5.40 ± 0.25	5.58 ± 0.20
Cinnamon	3.41^{c} ± 0.36	5.90 ± 0.27	5.90 ± 0.27
Oregano+Cinnamon	4.08^{ab} ± 0.19	5.33 ± 0.23	5.66 ± 0.20
Probiotic	2.49^{de} ± 0.37	5.30 ± 0.16	5.47 ± 0.08
Prebiotic	2.04^{ef} ± 0.03	5.58 ± 0.16	5.75 ± 0.12
Antibiotic+Enzyme	1.72^{f} ± 0.07	4.59 ± 0.38	5.26 ± 0.14
Oregano+Enzyme	1.89^{ef} ± 0.16	5.20 ± 0.37	5.53 ± 0.20
Cinnamon+Enzyme	2.14^{def} ± 0.01	5.10 ± 0.37	5.70 ± 0.14
Oregano+Cinnamon+Enzyme	2.19^{def} ± 0.03	5.80 ± 0.14	5.67 ± 0.20
Probiotic+Enzyme	1.92^{ef} ± 0.15	4.98 ± 0.25	5.38 ± 0.12
Prebiotic+Enzyme	1.73^{f} ± 0.05	5.09 ± 0.28	5.45 ± 0.16

[1] Values represent the average of three quail in each group at 35-d of age.
[a-f] Column means within the common supercripts do not differ (P >0.05).
s.e. - standard error.

The use of essential oils in combination with the enzyme complex, a probiotic and a mannanoligosaccharide with or without the enzyme complex in the wheat based diet positively significantly reduced the intestinal viscosity compared to the control diet.

No difference in performance and other parameters, except plasma total cholesterol and triglyceride levels and the intestinal viscosity studied in the current study, was observed among these novel feed additives. Therefore, further research is needed to find out the best solution. It is important to remember that factors like price of the product, the hygienic conditions of the farm, feed quality and flock status must be taken into consideration when evaluating the success of a product.

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