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Effect of Dietary Supplementation with Probiotic or Essential Oils on Growth Performance of Broiler Chickens

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Abstract: This experiment was conducted to investigate the effect of the dietary supplementation of a probiotic (*Lactobacillus plantarum* strain TN8) or a commercial blend of essential oils (clove and cinnamon essential oils) on growth performance of broiler chicken. The dietary treatments were: basal diet as control (C); basal diet plus probiotic (0,2% of *Lactobacillus plantarum* strain TN8 at 2×10^{10} cfu/g; P); basal diet plus essential oils blend (300 mg/kg; EOB). A total of 900 one-day old male broiler chicks were randomly allocated to 3 experimental groups of 6 replicates of 50 chicks each. The chicks were grown to 35 d of age. Body weight, feed intake and feed conversion were weekly determined. The mortality was recorded daily. Concerning weight gain, in the periods of 8-14, 15-21 and 22-28 days of age, the group fed the essential oils blend presented significantly ($p<0.05$) higher values as compared with the other treatments. No statistical difference was detected in feed intake among treatments in any of the evaluated age intervals. Feed conversion ratio differences were detected only in the second period of 8-14 days of age, with the group fed the essential oils blend presenting statistically ($p<0.05$) lesser FCR as compared with that fed the probiotic product, which were not different from the control group. Mortality was different ($p<0.05$) only in the periods of 0-7 and 8-14 days of age, which was higher in the control group as compared with that of the birds fed the probiotic product and those fed the essential oils blend. At the end of the rearing period (35 days of age), the results showed that the dietary supplementation with probiotic or essential oils led to a significant improvement ($p<0.05$) in body weight gain and a numerical, but non significant ($p>0.05$) improvement in feed conversion in relation to the control group. Based on these results it can be concluded that probiotic strain and essential oils dietary supplementation have a growth promoting effect on broilers.

Key words: Probiotic, essential oils, broiler, growth performance

INTRODUCTION

It is well known that antibiotics added to animal feeds as growth promoters allow intensive poultry production and improved feed conversion. However, this usage of antibiotic is possibly the most important factor that promotes the emergence and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine. Therefore it is forbidden in EU countries since January 2006 and in Tunisia since 2010. Because of that it was necessary to find other alternatives. There is a huge variety of products for replacing antibiotic growth promoters and probiotics and plant extracts, particularly essential oils, are among the candidates for their replacement.

Probiotics beneficially affect the host animal by improving its intestinal balance (Fuller, 1989). They create gut conditions that suppress harmful microorganisms and favor beneficial ones (Line *et al*., 1998; Mead, 2000). They have been shown to maintain health by reducing risk diseases, possibly through a reduction in proliferation of pathogenic species, maintaining microbiota balance in the gut (Line *et al*., 1998; Macfarlane and Cummings, 1999; Mountzouris *et al*., 2007), boosting immune function (Zulkifli *et al*., 2000; Kabir *et al*., 2004) and increasing resistance to infection (Herich *et al*., 1998; Aysigi *et al*., 2005; Rekiel *et al*., 2007). They have been also shown to improve the growth performance of poultry (Jin, 1997; Li *et al*., 2008).

Essential oils are plant extracts derived from herbs or spices, which have beneficial effect on animal production and health. A large variety o f the essential oils have properties which could potentially improve feed intake, digestion, feed conversion and body weight

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gain (Lovkova *et al*., 2001; Williams and Losa, 2001; Ertas *et al*., 2005). The mode of action of these feed additives is not completely clear. They have antimicrobial, antiviral, antioxidant and many other biological activities (Ertas *et al*., 2005; Cross *et al*., 2007). They act as an appetite and a digestibility enhancers through a stimulation of the endogenous digestive enzymes secretion (Williams and Losa (2001), Lee *et al*., 2003). These traits made essential oils a promising replacement of antibiotic growth promoters.

The objective of the current study was to examine the effects of dietary supplementation with probiotics or essential oils in broiler diets compared to standard broiler feed on the growth performance of broiler chicks.

MATERIALS AND METHODS

Animals and diets: Nine hundred day of hatch male broiler chicks (Arbor acres) were obtained from a local hatchery. All chicks were spray vaccinated for New Castle and Gumboro at the hatchery and no other vaccinations were administered.

Chicks were raised in floor pens with wood shaving litter. Six replicates of 30 chicks were considered for each treatment.

The broilers were fed a typical corn-soybean meal basal diet in mesh form (Table 1). The basal diet was formulated to meet or exceed NRC (1994) requirements for starter (1 to 14 d) and grower (15 to 35 d).

Chicks were randomly assigned to one of three dietary treatments. The dietary groups were: (1) basal diet without any additive (negative control, C), (2) basal diet supplemented with 2 g/kg of a probiotic (*Lactobacillus plantarum* strain TN8, P) and (3) basal diet supplemented with 300 mg/kg of an essential oils blend (clove and cinnamon essential oils, EOB). The diets were isocaloric and isonitrogenous. Feed and water supply were *ad libitum*.

The probiotic preparation (*Lactobacillus plantarum* strain TN8, 2×10^{10} cfu/g) was supplied by the Center of Biotechnology of Sfax - Tunisia and it was isolated from the crop of healthy adult chicken. The essential oils blend compromise clove and cinnamon essential oils and was purchased from Photosynthese (France).

Broilers were observed daily and any mortality was removed and body weight recorded. Broilers were weighed at 7, 14, 21, 28 and 35 d and body weight gain, feed consumption and feed conversion were weekly calculated. Body weights from mortality were used to adjust feed consumption.

Statistical analysis: The data were analyzed as a completely randomized design, using the General Linear Model (GLM) procedure of SAS (2001) and Duncan's Multiple Range test was used to detect ($p<0.05$) differences among treatment means.

Table 1: Ingredient composition and nutritional composition of basal diets

Ingredients and composition (%)	1 to 14d starter (%)	15 to 35d grower (%)
Corn	56.12	60.79
Soybean meal, 48	33	31
Vegetable oil/soybean oil	3	3
Limestone	0.73	0.78
Salt	0.35	0.35
Dicalcium phosphate	1.60	1.04
Methionine	0.24	0.20
Vitamin-mineral premix[1]	4	4
Total	100	100
Calculated analysis		
Metabolizable energy (kcal/kg)	2900	3050
Crude protein (%)	22.5	19
Lysin (%)	1.1	0.92
Methionine (%)	0.42	0.4
Methionine+cystine (%)	0.75	0.7
Calcium (%)	0.95	0.93
Available phosphorus (%)	0.45	0.42

[1]Vitamin-mineral premix (Alfa Nutrition Animal, Sfax, Tunisia), provides (per kg of diet): vitamin A (retinyl acetate), 12 188 IU; cholecalciferol, 2438 IU; vitamin E (DL-α-tocopheryl acetate), 18.3 IU; pantothenic acid, 42.6 mg; vitamin B_1, 1.2 mg; vitamin B_2, 7.3 mg; vitamin B_3, 9.7 mg; vitamin B_6, 1.2 mg; vitamin B_{12}, 0.024 mg; vitamin K_2, 1.2 mg; folic acid, 0.62 mg; choline chloride, 622.2 mg; calcium, 8784 mg; phosphorus, 3660 mg; sodium, 366 mg; magnesium 36.6 mg; iodine, 0.59 mg; cobalt, 0.59 mg; copper, 2.42 mg; iron, 45.75 mg; manganese, 97.36 mg; zinc, 85.39 mg; selenium, 0.11 mg; methionine, 1647 mg.

RESULTS AND DISCUSSION

The effects of dietary treatment on averaged daily weight gain, feed intake, FCR and mortality are shown in Table 2.

Average daily weight gain: In the period of 0-7 of age, no statistical differences between treatments were observed ($p<0.05$) for the daily weight gain. This parameter, in the subsequent three periods (7-14; 15-21 and 22-28 days of age), was higher in the group fed the EOB as compared with birds fed the probiotic product and the control group ($p<0.05$), which were not different. In the period of 29-35 days of age, the average daily weight gain of the two experimental groups were similar ($p<0.05$) and were not significantly different from the control group.

The improvement of body weight gain with the EOB treatment observed in this trial may be due to the active materials (cinnamaldehyde and eugenol) found in cinnamon, which are considered as digestion stimulating factors, in addition to their antimicrobial activity against pathogenic bacteria found in the intestine (Cabuk *et al*., 2003), thus resulting in greater efficiency in the utilization of feed and subsequently in enhanced growth. On the other hand there is a large number of studies which have not shown positive effect of using dietary EO supplementation on body weight gain (Vogt, 1990, 1991; Botsoglou *et al*., 2002;

Table 2: Effect of the experimental treatments on the growth performance of broilers at different periods of age[1]

	Treatments				
Item	Control	Probiotic	Essential oil blend	p-value	SEM
0-7 days					
DWG (g/bird/d)	19.44	18.87	19.91	0.3687	0.521
FI (g/bird/d)	22.50	21.82	23.04	0.0982	0.611
FCR (g/bird/d)	1.15	1.15	1.15	0.9993	0.000
Mortality (%)	1.00[b]	0.00[a]	0.00[a]	0.0251	0.577
8-14 days					
DWG (g/bird/d)	39.44[b]	40.00[b]	46.97[a]	0.0299	4.195
FI (g/bird/d)	56.73	55.90	58.23	0.2252	1.181
FCR (g/bird/d)	1.43[b]	1.39[b]	1.23[a]	0.0260	0.106
Mortality (%)	1.36[b]	0.00[a]	0.00[a]	0.0268	0.785
15-21 days					
DWG (g/bird/d)	55.83[b]	52.99[b]	58.47[a]	0.0356	2.741
FI (g/bird/d)	85.23	83.08	89.83	0.2107	3.448
FCR (g/bird/d)	1.52	1.56	1.53	0.3061	0.021
Mortality (%)	0.67	0.00	0.00	0.4219	0.387
22-28 days					
DWG (g/bird/d)	63.54[b]	64.17[b]	70.80[a]	0.0482	4.022
FI (g/bird/d)	109.56	110.78	116.88	0.2609	3.922
FCR (g/bird/d)	1.72	1.72	1.65	0.0933	0.040
Mortality (%)	0.00	0.00	0.00	0.7654	0.000
29-35 days					
DWG (g/bird/d)	71.88	74.76	75.50	0.2667	1.913
FI (g/bird/d)	134.67	139.83	140.07	0.0938	3.051
FCR (g/bird/d)	1.86	1.86	1.85	0.7513	0.006
Mortality (%)	2.07	2.00	1.35	0.8642	0.397

[1]Data are means and SEM (Standard Error Mean) of 6 replicates of 50 broilers per pen.
[ab]Means within a row with no common superscript differ significantly ($p<0.05$).

Garcia *et al*., 2007). Barreto *et al*. (2008) found that the essential oils of cinnamon and clove, individually added to broiler feed at 200 mg/kg, failed to influence broiler performance. The authors explained such an absence in significant treatment effects by a lack of microbiological challenges or inactivity of the added substances or perhaps due to the dose of the tested active principles. Isabel and Santos (2009) also failed to observe any effect on the body weight or average daily gain, for the whole experimental period (46 days), when a blend of clove and cinnamon essential oils (100 mg/kg) was added to the feed. Mukhtar (2011) carried out a study of 35 days to determine the effect of the dietary addition of different levels (200, 400 and 600 mg/kg) of clove oil on broiler performances. At the age of 35 days, the author did not note any significant difference for body weight gain in the experimental broilers when compared with those fed on negative control diet.

When poultry age is taken into account, the different responses in the current trial regarding the weight gain parameter may suggest that dietary EO supplementation is more efficient during the mid-starting and growing phases. These findings agree with the work of Al-Kassie (2009), who observed, when using an inclusion level of 100 mg/kg of EO derived from thyme or cinnamon, that during the period of 1-21 days of age, EO supplemented broilers had significantly higher body weight gain when compared with those of the negative control group, but the same did not occurred during the second period of measuring (22-42 age).

Without regards to the EO dose used in the present experiment and that employed by Al-Kassie (2009), results of the two studies seem to indicate that the efficacy or inefficacy of EO may be related to the birds age. It is well known that the younger birds are more susceptible to stress factors than the older birds. The presence of health challenges and stress in the rearing environment determine the efficacy of the EO. So this could explain why young birds responded more efficiently to the dietary EO supplementation than the older ones.

The lack of positive effect of probiotic treatment on broiler weight gain observed in this trial is in agreement with other studies (Watkins and Kratzer, 1984; Maiolino *et al*., 1992; Bitterncourt *et al*., 2011). On the contrary, there are many studies which have reported positive effects of using different strains of probiotics on this parameter (Jin *et al*., 1998; Zulkifli *et al*., 2000; Kabir *et al*., 2004; Li *et al*., 2008; Peric *et al*., 2010). These contradicting results indicate that the efficacy of probiotic application depends on many factors including species composition of probiotics, administration levels, application methods, overall diet composition, bird age and environmental factors as it has been suggested by Jin (1997), Jin *et al*. (1998) and Patterson and Brukholder (2003).

Feed intake: For the variable feed intake, no statistical differences among treatments were observed during the all periods of age, but chicks fed on diet supplemented with EOB showed numerically more in feed intake. These results are in line with those of Isabel and Santos (2009), Mukhtar (2011) and Aguilar *et al.* (2013) who did not found significant differences in feed consumption when evaluating EO as natural growth promoters in broilers feeding. In this study, the lack of significant difference in relation to the control diet could be explained by the fact that the poultry may not acutely respond to flavor as previously suggested by Moran (1982), although there is an evidence to suggest that herbs, spices and various plant extracts have appetite and digestion stimulating properties (Kamel, 2001). In this sense, Lee *et al.* (2004) have found that adding the cinnamon to the diet of broilers increased their feed consumption. Same result was also reported by Al-Kassie (2009) when they assessed different inclusion levels of essential oil in the diet. The authors found that extract oil derived from thyme and cinnamon in broiler diets improved significantly the feed intake. They attributed this effect to active principles (thymol, carvacrol, cinnamaldehyde and eugenol) present in these plants which are considered as digestion stimulating factors (Cabuk *et al.*, 2003).

The similar conclusion can be drawn for the addition of probiotic in broiler feed. The lack of influence of probiotic treatment on feed intake was also observed by Maiorka *et al.* (2001), when testing probiotic (*Bacillus subtilis*) product, as well as by Pelicano *et al.* (2004), who evaluated probiotics (*Bacillus subtilis* or blend of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus lactis*, *Streptococcus faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae*) and did not observe any difference in feed intake with the dietary incorporation of these products. However, other experiments have showed the effect of probiotics on the feed intake of broilers. Correa (2003) tested different probiotics in broiler diets (*Bacillus subtllis*, *Bacillus toyoi*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus salivarium*, *Streptococcus faecium* and *Saccharomyces cerevisiae*) and observed lower feed intake in the group fed one of the probiotics relative to the control group in the period of 0-21 days of age, which was also observed by Zulkifli *et al.* (2000). Boratto (2004), however, in the same period, found higher feed intake in broilers fed probiotics, in relation to the control group.

Feed conversion ratio: For FCR, statistical difference was detected only in the second experimental period (7-14 days of age), with the group fed the EOB presenting better FCR as compared with that fed the probiotic or the control group ($p<0.05$). Moreover, in the others evaluated age intervals, birds in EOB group recorded the lesser values of FCR. These results are consistent with those of other studies: Hernandez *et al.* (2004), Isabel and Santos (2009) and Sang-Oh *et al.* (2013) reported, respectively that he feed efficiency of broilers fed a diet supplemented with cinnamon, blend of clove and cinnamon or cinnamon powder was similar to that of broilers fed an unsupplemented diet. Barreto *et al.* (2008) found also that the essential oils of cinnamon and clove individually added to broiler feed at 200 mg/kg, failed to influence FCR. However, other experiments in which different EO were added to broilers diets have showed the effect of EO on the FCR of broilers (Tedesco, 2001; Kluth *et al.*, 2002; Alcicek *et al.*, 2003; Osman *et al.*, 2005). In theses studies the FCR-improving effect was attributed to a more efficient use of nutrients as suggested by Devriese *et al.* (1993) when they tried to explain the beneficial effect of growth promoter substances.

During all periods of measures, the probiotic treatment showed no positive effect on feed conversion ratio compared to the control group ($p>0.05$). This result is consistent with that of Perić *et al.* (2010), Rocha *et al.* (2010) and Bitterncourt *et al.* (2011) who did not detect statistical difference in feed conversion ratio among broilers group fed probiotic and control group in treatments in none of the studied intervals of age. Other authors, however, obtained better feed conversion ratio in broilers fed probiotics in the periods of 0-21 days (Corrêa *et al.*, 2003; Maiorka *et al.*, 2001; Pelicano *et al.*, 2004; Zulkifli *et al.*, 2000) and 0-40 days (Boratto *et al.*, 2004; Maiorka *et al.*, 2001).

Mortality: Mortality differences were observed only in the periods of 0-7, 8-14 days of age, with higher values in the control group as compared with the probiotic and the EOB groups ($p<0.05$). In addition, in the same periods, the group fed the probiotic was not different in relation to the group fed the EOB. Considering the other experimental periods, no influence of tested feed additives on mortality was detected.

On the use of probiotics in broilers diet, this results observed in this trial were similar to those found by Bitterncourt (2011) who found that mortality was different only in the period of 0-14 days of age and which was higher in the control group as compared with that of the birds fed the probiotic product, contrarily to the findings of Pelicano *et al.* (2004), who, in a study on the use of probiotics in broilers, observed at all experimental periods lesser mortality when these additives were added to the diet. Concerning the effect of the dietary supplementation of essential oils in broilers on mortality, responses observed in this experiment were in line with those of Mukhtar (2011). Authors found that chicks fed on diets contain clove oil recorded numerically the lowest rate of mortality compared to control group.

Table 3: Effect of the experimental treatments on the growth performance of broilers during the total rearing period (1-35 days of age)[1]

Item	Control	Probiotic	Essential oils blend	p-value	SEM
Initial weight (g/bird)	40.75	41.11	41.32	0.2799	0,288
Final weight (g/bird)	1721.67[c]	1796.67[b]	1886.80[a]	0.0147	82,680
Feed intake (g/bird)	2860.83[b]	2900.87[b]	2996.07[a]	0.0334	69,469
Weight gain (g/bird)	1680.92[c]	1755.56[b]	1845.48[a]	0.0051	82,398
Feed conversion ratio	1.70[a]	1.65[a]	1.62[a]	0.0999	0,040
Mortality (%)	5.03[b]	2.00[a]	1.35[a]	0.0019	1,964

[1]Data are means and SEM (Standard Error of Mean) of 6 replicates of 50 broilers per pen.

[abc]Means within a row with no common superscript differ significantly ($p<0.05$).

Considering the performances of broilers during the entire rearing period (1-35 days of age), obtained are shown in Table 3 shows the performance results of broilers during the entire rearing period (1-35 days of age). There were significant differences in final body weight, weight gain and feed intake between dietary treatments ($p<0.05$). The birds fed on diet supplemented with the EOB presented higher final weight, weight gain and feed intake as compared with the other broilers (control and probiotic groups). The birds receiving the diet containing the probiotic had a higher final weight and weight gain than those of the control group ($p<0.05$), but difference in feed intake was not significant between the two groups. No statistical difference was detected in feed conversion ratio among the three dietary treatments during the whole experimental period. However birds fed on diet without additives (probiotic/EOB) recorded numerically the worse FCR. For mortality, broilers fed on diet containing probiotic or EOB had similar mortality rate, which was significantly lower than that of the control broilers.

These results agree partially with the work of Al-Kassie (2009), who found that the supplementation of 200 mg/kg oil extract derived cinnamon in broiler diets significantly improved the overall growth performance traits (weight gain, feed intake and FCR) as compared to control broiler during a growing period of 6 weeks. Indeed, in the present experiment which was carried out for 5 weeks only, difference in the overall FCR was not significant. Nevertheless birds fed on diet containing EOB recorded numerically the lowest FCR. The significant improvement of body weight gain and feed intake and the slight amelioration of FCR may be due to the active materials (Cinnamaldehyde and eugenol) found in cinnamon, causing greater efficiency in the utilization of feed, resulting in enhanced growth. There is an evidence to suggest that herbs, spices and various plant extracts have appetite and digestion stimulating properties (Kamel, 2001). These results agree with the work of Lee *et al*. (2004).

Based on these results it can be concluded that probiotic strain (*Lactobacillus plantarum* strain TN8) and clove and cinnamon essential oils under investigation can enhance growth performance when these additives are added to the animals feed.

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