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Full Length Research Paper

An investigation on the replacement of antibiotics by medicinal plants to control the infection of *Escherichia Coli* (*E. coli*) in broiler chickens

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This study was done to determine the effects of garlic, mint and onion in feeding of the broiler chickens as a growth natural factor (GNF) instead of antibiotics. First, the antibacterial effects and minimum inhibitory concentration (MIC) of 0.5, 1, 2.5, 5, 10 and 20% concentrations of aqueous extracts of garlic (*Allium sativum*), mint (*Mentha* spp.) and onion (*Allium cepa*) on *E. coli* were determined in *in vitro* conditions. Results show that the minimum inhibitory concentration (MIC) of garlic extract was 0.5%. Distilled water, phenol phenicol and Floxacin were used as solvent and control. In the next step, the effects of adding garlic to the diet of broiler chickens were analyzed at farm. 300 day-old broilers (Arbor Acres Plus) were divided into groups of 60 birds each and randomly assigned to the five treatment diets. Each treatment has three replicates. These chickens were kept up to the age of 56 days (8 weeks), all under study and fed with the standard ration. According to the corrections, adding 8% garlic to the diet was equal to the minimum inhibitory concentration (MIC) in the lab. As a result, we used garlic itself instead of its aqueous extract in the diet. The different experimental groups did not receive any antibiotic and growth additives. The different experimental groups were as follows. The 1st group which was considered as the control group received feed without garlic. The 2nd group, one day in week, during breeding period received feed containing 8% garlic. The 3rd group, 2 days in week, during breeding period received feed containing 8% garlic. The 4th group, one day in week and in the 1st, 4th, 5th, 6th, 7th and 8th weeks, received feed containing 8% garlic. The 5th group, 2 days in week and in the 1st, 4th, 6th, 7th, and 8th weeks, received feed containing 8% garlic. During the conduct of the study, the performance of broiler chickens was recorded and then analyzed statistically. In the end, the results show that adding 8% of garlic to the feed of broiler chickens, in the probability level of 5% statistically did not have a significant effect on feed intake, body weight gain, feed conversion ratio, the mean of chicken's weight, mortality percentage, dressing percentage, offal percentage, abdominal fat weight, weight of liver, spleen, pancreas, cecum, leg and breast and cecum and intestine size and taste (p>0.05), although, there was a little difference in the case of feed intake, weight gain, mortality percentage, intestine size and taste among different experimental groups. As a result, groups that consumed garlic showed a better performance.

Key words: Medicinal plants, aqueous extracts, garlic, mint, onion, antibiotic, *E. coli*, broiler chickens, performance.

INTRODUCTION

The disease of *Collibacillos* is one of the widespread and major diseases seen in industrial poultry houses. The cause of the disease is *Escherichia Coli* (*E. coli*), a Gram-negative bacterium and intestinal flora of human, animal and poultry, which in some conditions becomes pathogenic in the birds. This bacterium is transmitted to birds through faeces, water and dust. When the birds' resistance declines, it becomes pathogenic and the disease widely spreads among the broilers, subsequently resulting in lowering the eggs quality, high economic costs and infection of yolk sac, respiratory system injury, digestive disorders in intestine, etc. One of the causes for

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the inefficiency of antibiotics and chemical drugs in *Collibacillos* disease treatment is that the drugs do not reach air sacs, lungs and cardiac membranes. In addition, bacteria may be resistant to drugs which have been used repeatedly. But the use of herbal drugs and derivatives can reduce the aforementioned effects because the effective materials in plants have got a kind of biological balance and create fewer side effects in the body.

There are different reports about the use of onion and garlic aqueous extracts (Shams et al., 2003), the antifungal effects of essential oils and herbal extracts (Wilson et al., 1997), the effect of garlic extract on chickens (Hidajati, 2005), the antimicrobial effect of onion and garlic extracts (Elnima et al., 1983), the effect of garlic essential oil on the poultry intestinal bacteria (Zennere et al., 2003), the antifungal and antibacterial activity of *Senecio* plant (Loizze et al., 2004), the effect of the mixture of herbal extracts on the broiler chickens infected with *Eimeria tenella* (Christaki et al., 2004) and the effect of dietary herbal supplements on the quality of fresh and frozen meat (Gardzielewska et al., 2003).

The purpose of this experiment was, on one hand, to investigate the antibacterial effects and to determine the minimum inhibitory concentration (MIC) of aqueous extracts of garlic (*Allium sativum*), mint (*Mentha* SPP.) and onion (*Allium cepa*) on *E. coli*, and on the other hand, to add garlic to diet of the broiler chickens in order to prevent and cure the infections of *E. coli* and to improve the performance of the chickens during the breeding period, as well as introducing the herbal extracts as a growth natural factor (GNF) instead of antibiotics.

MATERIALS AND METHODS

In vitro conditions

The first stage of this experiment was performed *in vitro*. Samples were isolated from the infectious tissues of the chickens and cultured on the Eosin methylene blue (E.M.B) agar medium which was incubated at 37°C for 24 h. Then 9 cavities were prepared in plates containing nutrient agar medium by a 5 mm diameter sterile drill (6 cavities for the herbal extracts, one cavity for distilled water and the two other cavities for *Phenol Phenicol* and *Floxacin* as control). After that the plates were incubated for 24 h. Next, plates containing E.M.B agar medium were taken out and 5 to 6 *E. coli* colonies (metallic sheen) were cultured on nutrient agar medium by sterile swab; 5 ml of the 6 concentrations for aqueous extracts (garlic, onion and mint) were poured in the cavities by sampler and the plates were kept in incubator at 37°C for 24 h. Finally, the plates were taken out and the zone of inhibition was measured by scale. *Phenol Phenicol* and *Floxacin* were used as control. This test was repeated 50 times.

In farm (rearing)

Considering the obtained *in vitro* data conditions, at this stage, the effect of adding 8% garlic in broiler chickens diet was evaluated. It is noteworthy, that 8% garlic in diet was an equivalent for MIC in laboratory conditions. The 300 day-old broilers (Arbor Acres Plus) were divided into five treatment groups of 60 birds each and randomly assigned to the five treatment diets. Each treatment group was further sub-divided into three replicates of 20 birds per replicate. The breeding house was partitioned into 15 parts (cages) of 3 square meters enclosed by iron nets. All the chickens under the study were fed with the same standard diet. The feeding method was followed based on the NRC recommendation and based on its tables in 8 phases (8 weeks and with a certain diet for each week). No antibiotics were added to the feed. According to the fact that *in vitro* conditions MIC for aqueous extract of garlic was 5% and that we had to add garlic itself to the diet, regarding some corrections made on the proportion, 8% garlic was added to the diet. Garlic was added to all groups feed except the control group. The different experimental groups were as follow:

Group 1: Control group, received standard diet without garlic
Group 2: Once a week, during breeding period, received feed containing 8% garlic
Group 3: Twice a week, during breeding period, received feed containing 8% garlic
Group 4: Once a week, in the 1st, 4th, 5th, 6th, 7th and 8th weeks, received feed containing 8% garlic
Group 5: Twice a week, in the 1st, 4th, 5th, 6th, 7th and 8th weeks, received feed containing 8% garlic

Data collected were subjected to analysis of variance, and where significant differences were observed, means were further subjected to Duncan's multiple range test (MRT), SPSS and Mini Tab software (2004, 2003). The results were considered as significant when p values were less than 0.05 ($p<0.05$).

Parameters of performance

Both in the beginning and during the breeding period, all the chickens under the study were weighed. Feed conversion ratio and body weight gain were calculated on a weekly basis. Feed intake and mortality percentage were recorded for each group each day. At the end of the breeding period, one chicken with the nearest mean weight of that unit was separated and killed. Then, dressing percentage, abdominal fat weight, weight of liver, spleen, pancreas and cecum, intestine and cecum size, offal percentage, weight of breast and leg were measured.

RESULTS AND DISCUSSION

Findings show that aqueous extract of garlic has inhibition effect on the growth rate of *E. coli*, and the minimum inhibitory concentrations (MIC) were 5%. Based on the given results (Tables 1, 2 and 3), adding 8% garlic to the feed of different experimental groups did not have significant difference on the performance of broiler chickens ($p>0.05$), whereas there was a little difference among different experimental groups.

Although, the addition of 8% garlic did not have any significant effect at 5% probable level on the broiler chickens' performance, little difference was among different experimental groups. For example, in relation with feed intake, group 2 showed a significant difference with group 1, which was the control group that can be related to stimulation of appetite by garlic. Group 2 had more increase of body weight gain as compared to that of group 1 that can be related to the more intake of garlic

Table 1. Comparison of the means of feed intake, body weight gain, feed conversion ratio, the mean of chicken's weight and mortality percentage of broiler chickens with different experimental groups in the breeding period (1 to 56 days) by Duncan method.

Group	Feed intake (g)	Body weight gain (g)	Feed conversion ratio (%)	Mean chickens' weight (g)	Mortality percentage (%)
1	4698.83[a]	1997.00[ab]	2. 41[a]	2068.00[ab]	10.00[a]
2	5770.67[c]	2379.67[b]	2.44[a]	2434.33[b]	13.33[a]
3	11.67[a]	2425.33[b]	2.39[a]	2366.67[b]	5672.67[bc]
4	18.33[a]	1825.67[a]	2.88[a]	1764.50[a]	5040.17[ab]
5	3.33[a]	2199.67[ab]	2.37[a]	2082.33[ab]	934.17[a]

Common letter in each column indicates non- significant difference and uncommon letter in each column indicates presence of significant different at the probable level of 5%.

Table 2. Comparison the means of carcass characteristics of broiler chickens in different experimental groups at the end of breeding period (56 days) by Duncan method (%).

Group	Dressing percentage	Weight of abdominal fat	Weight of liver and spleen	Weight of full gut	Weight of empty gut
1	70.72[a]	1.13[a]	2.91[a]	12.80[a]	8.95[a]
2	69.71[a]	1.10[a]	2.14[a]	11.44[a]	7.93[a]
3	67.17[a]	1.21[a]	2.18[a]	10.86[a]	8.41[a]
4	60.43[a]	1.05[a]	2.45[a]	13.68[a]	9.56[a]
5	60.82[a]	1.02[a]	2.41[a]	11.91[a]	8.81[a]

Common letter in each column indicates non- significant difference at the probable level of 5%.

Table 3. Comparison the means of carcass characteristics in broiler chickens with different experimental groups at the end of breeding period (56 days) by Duncan method (%).

Group	Cecum weight	Pancreas weight	Intestine length	Cecum length	Breast weight	Leg weight
1	1.02[a]	0.2397[a]	0.1231[b]	0.0135[a]	25.81[a]	24.58[a]
2	0.7137[a]	0.2290[a]	0.0761[a]	0.0115[a]	26.57[a]	32.51[a]
3	1.04[a]	0.2160[a]	0.0812[a]	0.0104[a]	26.68[a]	25.44[a]
4	0.8970[a]	0.1853[a]	0.1334[b]	0.0141[a]	26.68[a]	30.41[a]
5	0.9433[a]	0.1863[a]	0.0959[a]	0.0131[a]	28.86[a]	31.24[a]

Common letter in each column indicates non-significant difference at the probable level of 5%.

containing feed which causes better digestion and absorption (Elsom et al., 2003). As to feed conversion ratio, there was no significant difference between the different experimental groups. It shows that adding garlic to the diet did not affect the feed conversion ratio. Concerning the mean of chicken's weight, the second treatment showed a greater mean of weight compared with the first treatment, which was the control group. This is probably due to a greater feed intake in groups which consumed garlic-containing feed.

The results presented in Table 1 indicate that percentage of mortality has minor difference among the different experimental groups, but it was significant in groups 4 and 5. Although, there was a minor difference among the groups about carcass yield, the results in Table 2 showed that group 1, as control group, had the highest carcass efficiency. The obtained results reveal that the addition of garlic to the diet had no effect on the carcass efficiency. There was no significant difference about the abdominal fat weight among the groups. But group 1 had a higher abdominal fat weight that indicates adding garlic to the diet helps the digestion of fat more considerably.

No significant difference was observed on the percentage of liver and spleen weight compared with the control group, although, group 1, which was the control group, had the highest percentage of liver and spleen weight. These findings show that supplementing 8% garlic to diet of broiler chickens has no toxic and inflammatory effect; therefore it does not lead to inflammation and increase in liver and spleen weight. As a result, garlic has no toxic and inflammatory effect on the birds.

Results in Table 2 showed that percentage of full and

empty gut weight among the groups had minor differences. In this regard, groups 2, 3 and 5 as compared to the group 1 had less weight of full gut, which may be due to killing of intestinal micro-organisms by garlic. Regarding the empty gut, group 1 had more weight. There was minor difference between weights of cecum. Groups 2, 4 and 5, as compared with the group 1 had less cecum weight that is probably because of antimicrobial effect of garlic which caused the destruction of bacteria in cecum. As a result, the percentage of cecum weight in groups receiving garlic was less.

Table 3 shows that the percentage of pancreas weight among different experimental groups had little difference; the control group (group 1) had higher pancreas weight compared with other groups. This shows that adding 8% garlic to the diet has no toxic and inflammatory effect. There was minor difference between the percentage of intestine and cecum length. Considering this, group 4 had longer intestine and cecum compared with other groups. This table also reveals that the percentage of breast and leg weight had minor difference in the groups, but the control group had less breast and leg weight. It seems that garlic-containing feed increases fat digestion and reduces the meat fat, thereby increasing the percentage of net meat.

Conclusion

Although, this finding is not similar to the reports given by Ciftci et al. (2005), which indicated that adding anise essential oil to the broiler chickens diet causes 15% increase of daily body weight gain of the chickens and the feed conversion ratio by 12% as compared with the control group. Also, in reports by Ertas et al. (2005), adding a mixture of essential oil of anise, clove and oregano to the diet of broiler chickens at the level of 200 ppm improved the daily body weight gain by 16% and feed conversion ratio by 12%, as compared with that of the control group. However, our results are similar to the reports given by Christaki et al. (2004), indicating that supplementing Apacox plant to the diet of broiler chickens infected with *Eimeria tenella* reduced feed conversion ratio as compared with the control group.

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