REDUCTION OF BROILERS INTESTINAL PATHOGENIC MICRO-FLORA UNDER NORMAL OR STRESSED CONDITION

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Abstract: *This study was conducted to determine the effect of Organic acid (citric acid), prebiotic (lactose) and aromatic plant (rosemary), added to a standard diet, to reduce or control the prevalence pathogenic bacteria and parasite in the intestine of broiler chicks reared under normal or heat stress conditions. Two hundred forty, unsexed one-week-old, Hubbard chicks were divided into groups of 60 birds each and randomly assigned to the four treatments. Each treatment has two replicates. Experiment was as follow: A control group with no supplement and the other three groups were supplemented with one of the following additives; citric acid, lactose or rosemary. At 35 days of age, each group was divided into two equal sub-groups, the first was kept under normal conditions (23°C) while, the second was exposed to 38°C for 3 hrs daily for 6 consecutive days from 35 to 40 days of age with 70 % relative humidity.*

The three experimental additives (citric acid, lactose and rosemary) had statistical effects (P<0.05) regarding the decrease in the counts of pathogenic intestinal bacteria (i.e., total aerobic bacteria, E. coli, salmonella and staphylococci) and parasite (coccidia ovum) in ileum, caecum or fecal matter under normal temperature (23°C) or high temperature (38°C) compared to the control groups which received diets without experimental additives. Furthermore, they significantly (P<0.05) improved body weight gain, mortality rate, feed consumption, feed conversion and carcass characteristics of broilers. The addition of the dietary rosemary had a positive (P<0.05) effect on total proteins (albumin and globulin), lymphoid organs relative weights (bursa and thymus), Leukocytic cells (WBC's) and Heterophil cells either under normal or high temperature conditions. Conversely, it has negative (P<0.05) effects on the total lipids, cholesterol and Monocyte cells of broilers blood. However, T3, creatinine, AST and ALT enzymes, hemoglobin concentration and hematocrit values were not affected by any of the three experimental additives. There were decreases (P<0.05) in body weight gain, feed *consumption, (T3), plasma total proteins and increase in mortality rate of chicks subjected to heat stress. However, experimental treatments reduced the deleterious effects of heat stress. Citric acid, lactose and rosemary were efficient as antibacterial and bacteriostatic activities in reducing or controlling the intestinal pathogenic bacteria and parasite, consequently, improving the broiler production and physiological status. Therefore, it is advisable to use organic acid (citric acid), prebiotics (lactose) or aromatic plant (rosemary) in broiler diets under either normal or stressed environmental conditions.*

INTRODUCTION

Chickens are more susceptible to colonization by pathogenic bacteria when they are newly hatched or after times of stress (immature and/or disrupted intestinal micro-flora). The intestinal tracts of chickens are well established as reservoirs for pathogenic avian E. coli in association with the intestinal micro-flora of healthy birds, but only cause disease in case of secondary environmental and host predisposing factors (Moulin and Fairbrother, 1999).

Organic acids have been studied as a tool to reduce undesirable bacteria during poultry production. Organic acids could control and limit the growth and colonization of numerous pathogenic and nonpathogenic species of bacteria in the gut (Hinton and Linton, 1998) they also stated that it could increase digestibility of protein and regulate the intestinal microflora. Citric acid has been reported to inhibit bacterial growth (Roe *et al.*, 1998). Organic acids are recognized as one of the best alternative of antibacterial growth promoter in broiler feed or drinking water. Significant improvement in performance of broilers fed diets supplemented with propionic or fumaric acids were reported by Waldroup et al., (1995). Also, Inclusion of organic acids into chicken diets altered the populations of intestinal microflora and reduced the total number of coliforms bacteria and microbial total count (Waldroup et al., 1995 and Griggs and Jacob 2005).

According to the definition by Gibson and Roberfroid (1995), Prebiotic is a nondigestible food ingredient that affects the host by selectively stimulate the growth and / or activity of one or a limited number of beneficial bacterial species already resident in the colon. Lactose sugar is well documented as a prebiotic for poultry (Kermanshahi and Rostami; 2006). Beneficial bacteria could provide both a health benefit to the animal such as reduction of pathogenic bacteria in the animal. Hence, the fact that birds are incapable of enzymatic lactose digestion makes lactose a potential prebiotic agent. Some prebiotics are thought to enhance the growth of beneficial organisms in the gut, and others are thought to function as competitive attachment sites for pathogenic bacteria. Prevention of Salmonella or bacterial colonization in chickens can be achieved by feeding prebiotics (Patterson and Burkholder, 2003). Lactose has been shown to protect against Salmonella in chickens in several studies (Ziprin et al., 1991; Tellez et al., 1993; Corrier et al., 1997). Several studies have reported that E. coli and Salmonellae and other pathogens were inhibited due to adding lactose into poultry diets (Oyofo et al 1989 and Chambers et al, 1997). Also, Several experiments have stated that prebiotics can improve the body weight in broilers (Yusrizal and Chen, 2003).

Nowadays, there is a great interest to new sources of natural antioxidant and antimicrobial agents. Rosemary as an aromatic plant has considerable attention because of its potential antioxidant and antimicrobial properties (Moreira 2005 and Proestos et al. 2006). Rosemary has been used traditionally in therapy of some diseases for a long time. Rosemary essential oil is used as an antibacterial and antifungal (Oluwatuyi et al., 2004; Fernández, 2005). Antibacterial (Gram-positive bacteria Staphylococcus aureus and Bacillus cereus and Gram negative bacteria E. coli and Pseudomonas aeruginosa), antifungal (Candida albicans) activities (Kabouche 2005) and antioxidant activities (Genena et al., (2008) of rosemary extracts were confirmed. Rosemary have been used as stimulating effects of digestion and antiparasitic and antibacterial on *E. coli* and *Salmonella typhimurium* (Singh *et al.*, 2002; Cabuk *et al.*, 2003; Tabancal *et al.*, 2003). Also, it can be used as antifungal agent (Soliman and Badea 2002).

The present study has focused on reducing or controlling the prevalence pathogenic bacteria in intestine of broiler chicks under normal and heat stress conditions. Subsequently, improve the productive and physiological performance of these birds. Organic acids (citric acid), prebiotic (lactose) or herbal plants (rosemary) have been used to achieve these goals.

MATERIALS AND METHODS

The present study was carried out at Gimmizah Poultry Breeding Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Gharbiyah Governorate, Egypt, during winter season months, December and January.

Experimental birds:

Two hundreds and forty, one-day-old, unsexed broiler chicks (Hubbard) obtained from a commercial hatchery. At one week of age, chicks were distributed randomly and divided equally into four experimental groups, nearly equal in average live weight. Each group was represented by birds in two replicate floor pens of 30 chicks each, and kept under similar management conditions.

Treatments:

Chicks were treated as follow: The first was untreated group (control). While, the second group was treated with citric acid (2g/kg feed) from the beginning till the end of the experiment. The third was received 2 g lactose / kg feed through the experimental period. Finally, the fourth group was fed control diet inoculated with dried grounds of 1% rosemary through the experimental period. Diets and water were provided *ad libitum* under light cycle 23L: 1D throughout the experimental period. At 35 days of age, each group was divided into equal two sub-groups of fifteen birds each. First: remained on the same normal conditions (23°C), whereas, the second was exposed to 38°C for 3 hrs daily for 6 days from 35 to 40 days of age with 70% relative humidity.

Standard diets:

The control diets were formulated to meet the nutrient requirements of the chicks according to the strain catalog recommendation and their composition is shown in Table (1). Two type of diets were used, the starter and the finisher diets. The starter was fed for the first 3 weeks (2-4 weeks) followed by the finisher for the rest of the experiment which lasted for 42 days.

Measurements:

Live body weight and feed consumption were biweekly recorded and body weight gain and feed conversion were then calculated. Mortality was recorded daily throughout the experiment period.

Sampling and analysis:

At the end of the study, 5 birds from each treatment were slaughtered and two blood samples were collected from each bird in heparinized tubes, first one, centrifuged at speed 3000 rpm for 15 minutes and the plasma was stored at -20°C for later analysis. Hematocrit (Ht) value and hemoglobin (Hb) level were determined. Commercial Kits were used to determine Tri-iodothyronine (T_3) (ng/dl), total protein (g/dl), albumin (g/dl), alanine transaminase (ALT) (U/L), aspartate transaminase (AST) (U/L), total cholesterol (mg/dl) and total lipids (mg/dl) according to the manufacture recommendations of commercial kits. Globulin was calculated by substraction of plasma albumin from total protein. While, the second blood samples were used for detection of the total red blood cells (RBC's) count/mm^3 blood, total white blood cells (WBC's) count/mm^3 blood and their differential. Moreover, lymphoid organs (thymus and Bursa of Fabricius glands) were also weighed and presented as relative body weight (mg/100g body weight).

Microbiological study:

At the time of slaughter test, 5 samples of ileum and caecum contents were collected and examined to define and count the Pathogenic bacteria for each treatment. Fecal matter samples were collected in sterile polyethylene bags. All samples were delivered directly to the laboratory for bacterial count and definition using the procedure of A.O.A.C. (1990).

Statistical analysis:

Data were subjected to two-way analysis of variance using **(SAS Institute 1998).** The statistical model used for analyzing data obtained was: Yijk = M + Ti + Hj + (TH)ij + Eijk. Where, Yijk = The observation for each dependent variable; M= The overall mean; Ti =The treatment effects; Hj = The heat effects; (TH)ij = The effects of interaction between treatments and heat (1, 2,......, and 8); Eijk = The experimental error. Significant differences among means of interaction between treatments and heat were separated using Duncan (1955) Multiple Range Test. The percentage values were transferred to percentage angle using arcsine equation before subjected to statistical analysis, and then actual means are presented.

RESULTS AND DISCUSSION

Broiler performance:

Weight gain:

At 6 weeks of age, the different experimental treatments showed a significant ($P<0.05$) improvement in body weight gain compared to the control groups which received diets without experimental additives either under normal (23°C) or high temperature (23°C) conditions. Weight gain was increased by 8.85%, 7.82% and 8.09% at normal temperature and 7.06%, 7.62% and by 8.29% at high temperature when feeding citric acid, lactose and rosemary, respectively (Table 2).

Mortality rate:

Significant improvements were detected due to feeding diets supplemented with experimental additives at normal (23°C) from 7 to 35 days of age compared to the control group (Table 2). The same trend was observed during high temperature (38°C) at 35 to 40 days of age. Generally, improvements were in the following order: rosemary, citric acid and lactose at both conditions.

Consumption and conversion of feed:

Results indicated that under normal (23°C) or high temperature (38°C) conditions, the average values of feed consumption (g/chick) was ($P<0.05$) increased and feed conversion (g feed/g gain) was ($P<0.05$) improved due to using the citric acid, lactose and rosemary compared to control groups. Feed conversions were improved from 2.28 in control group to 2.24, 2.22 and 2.21 at normal temperature (23°C) or from 2.21 in heat stressed control group to 2.18. 2.11 and 2.15 at high temperature (38°C) with using the same additives (Table 2).

Carcass characteristics:

Significant improvements ($P<0.05$) were detected in relative weight of carcass, giblets and dressing of birds fed experimental additives (Table 2). Where, the lowest values were found with control groups at 6 weeks of age under normal temperature (23°C) or high temperature (38°C).

Lymphoid organs:

The data in Table 2 showed that the rosemary had statistical effect ($P<0.05$) regarding the increase in the relative weight of bursa and thymus compared with the control ones either under normal (23°C) or high temperature (23°C) conditions. In general, increasing the relative weights of both bursa and thymus due to experimental additives may reflect higher immunity. However, the other two experimental additives did not result any significant effects in tested lymphoid organs.

Present results showed in (Table 2) indicated that, the deleterious effects of high temperature on performance parameters were significant ($P<0.05$) improved by experimental additives inclusion.

The improvement in body weight gain of birds fed citric acid may be due to the utilization of minerals that positively affecting the growth (Abd El-Hakim et al., 2009). Dietary citric may decrease intestinal pH and increase calcium availability (Snow *et al.*, 2004), utilization phytate phosphorus and trace minerals (Boling *et al.*, 2000). Organic acids could increase digestibility of protein and regulate the microflora in the gut (Hinton and Linton. 1998) who added that citric acid could be used as growth promoter for chicken to alleviate the detrimental effects of heat stress. Significant improvement in performance of broilers (weight gain, mortality rate, conversion of feed and relative weight of carcass) fed diets supplemented with Citric, propionic or fumaric acids were reported by Snow *et al.*, 2004, Ali *et al.*, 2008 and Abd El-Hakim et al., 2009).

Addition of lactose has been shown to change the intestinal microflora and suppress the undesirable bacteria (Bailey, 1991 and Gibson *et al.*, 1995), stimulate mineral absorption, mainly calcium and magnesium (Scholz-Ahrens and Schrezenmeir, 2002). Gibson and Roberfroid (1995) indicated that prebiotic can beneficially affect the host by selectively stimulating the growth and/or activity of healthy bacteria in the colon. Also, the improvement in mortality or weight gain may be due to the increase in feed intake, utilization of nutrients and crude fiber and metabolizable energy apparent digestibility as reported by (Oyarzabal and Conner 1995 and Patterson and Burkholder, 2003) who added that dietary prebiotic were able to improve performance (Body weight gain and mortality rate) in broiler chickens and to limit *Salmonella* incidence in broiler gut. Lactose improved ($P \leq 0.05$) the crude fiber apparent digestibility and metabolizable energy compared with that in the control group (Barnhart et al., 1999, Sales and Janssens, 2003 and Giedrius et al., (2008).

Improvement in performance of broilers (weight gain, mortality rate and conversion of feed) fed diets supplemented with rosemary was confirmed by Singletary and Rokusek (1999) and Radwan (2003). They supplied 0.5 % rosemary and found an improvement in digestibility of most nutrients, except the ether extract which was decreased without affecting the metabolizable energy value compared to control. These improvements could be attributed to presence of phenolic diterpenes, flavonoids, trepioneol, carvophyllene, thujene, copalene, methyl chavicol, thymol and phenolic acids that had antimicrobial, antioxidant, improving nutrient utilization and growth stimulant effects (Lopez-Bote et al., 1998).

Blood biochemical:

Feeding broiler diets containing tested experimental additives has not any significant effect at day 40 of age blood biochemical items listed in Table (3) either under normal or heat stress conditions. Except the totals of lipids and cholesterol were significant ($P<0.05$) decreased. Also, total proteins as well as albumin and globulin for group fed diets contain rosemary were increased (P<0.05) as compared with control group. A result of total lipids and cholesterol reflects the hypocholestrolemic and hypolipidemic properties of experimental additives. Similarly, El-Deep et al., (2006), Radwan (2003) and (Ali *et al.*, 2008) reported that total of cholesterol and lipids were significantly decreased due to feeding a diet containing 1% rosemary to broilers. Also, they added that AST and ALT were not affected. Diets containing rosemary decreased intestinal absorption of lipids (El-Deep et al., 2006) and did not improve digestibility of ether extract (Radwan 2003) compared to control ones. No difference was observed in the total protein, albumen or globulin in the plasma of broilers fed diet supplemented with citric acid, lactic acid and their combinations (Ali *et al.*, 2008 and Abd El-Hakim et al., 2009) or fructooligosaccharides and fructans as prebiotics (Yusrizal and Chen 2003)

Increasing blood constituents in heat stressed groups compared to unstressed groups may attribute to the alteration of the blood volume as a result to evaporation from respiratory system during panting process.

Hematological picture:

Data in Table (4) indicates that experimental additives did not significantly affect the erythrocytic (RBC's), Leukocytic (WBC's) and their differential counts in terms of Lymphocytes, Eosinophil, and Basophil either under normal or heat stress conditions. Except the rosemary caused increases (P<0.05) Leukocytic (WBC's) and Heterophil cells, in contrary, Monocyte was decreased (P<0.05) under normal temperature (23°C) or high temperature (38°C) compared to the control birds at 40 days of age. Similar results were stated by Craig (1999) stated that several herbs can help providing some protection against bacteria and stimulate the immune system. Recently, Mahmoud et al., (2008) who fed 0.1 and 0.2% rosemary to grown male Gemmizah and noted (P<0.05) increased Leukocytic cells (WBC's), insignificantly differences in Lymphocytes, decrease in Monocyte cells and significantly higher values of Eosinophil.

Effects of feeding the citric acid, lactose and rosemary to broiler under normal or high temperature conditions on blood hematology are very rarely and need further studies.

Bacteria Enumeration:

Results showed that using experimental feed additives in broiler diets caused severe suppression regarding the pathogenic intestinal bacteria or parasite counts (Table 5). Where, the great effects were on the counts of total aerobic bacteria, E. coli, salmonella, staphylococci and Coccidia ovum in ileum, caecum or fecal matter compared to the control group which received diets without experimental additives. This may be considered as an indication to the fact that the addition of the citric acid, lactose or rosemary led to a severe decrease in the pathogenic intestinal bacteria counts. Total aerobic bacteria and E. coli counts in the ileum at normal temperature (23°C) was reduced from $7.02x10^6$ and ($6.31x10^6$) in control group to $3.85x10^6$ and $5.19x10^6$ in citric acid treatment by about 45.16% and 17.75%. Also, reduced to 5.36 $x10^6$ and 5.87 $x10^6$ in lactose treatment was about 23.65% and 6.97%. Also, reduced to 4.18 $x10^6$ and 4.94 $x10^6$ in rosemary treatment was about 40.45% and 21.71%. Also, the same trend was observed in all type of tested bacteria or conditions of experiment. Moreover, salmonella and Coccidia ovum were approximately absent in the ileum, Caecum and faeces at either temperature conditions.

Concerning citric acid results, pathogenic species of bacteria in ileum and caecum were reduced or their growths were limited as affected by citric acid addition. Similar studies reveal unequivocal conclusions (Waldroup et al., 1995, Roe *et al*., 1998, Griggs and Jacob 2005 and Ali *et al.,* 2008). They stated that inclusion of organic acids into chicken diets altered the populations of intestinal microflora and reduced the total number of coliforms bacteria and microbial total counts. Citric acid has been reported to inhibit bacterial growth (Roe *et al*., 1998). It can disrupt the membrane integrity of the organic matter of gram-negative bacteria affecting their survival (Ocana-Morgner and Dankert, 2001). The key basic principle on the mode of action of organic acids on bacteria is that nondissociated (non-ionised, more lipophilic) organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria (Dhawale, 2005).

Several experiments have demonstrated that prevention of Salmonella colonization in chickens can be achieved by feeding prebiotics (FOS and lactose). Likewise, Oyofo et al. (1989), Bailey, (1991), Gibson *et al*., (1995), Corrier et al. (1997), Barnhart et al. (1999) and Patterson and Burkholder, (2003), all of them stated that lactose addition has been shown to change the intestinal microflora and suppress the undesirable bacteria. Bailey (1991) found that prebiotics (FOS and lactose) acted against *Salmonella* when broiler chicks were stressed by feed and water removal for 24 h. They added that *Salmonella* was unable to grow when prebiotics was substrate. It has been hypothesized by Oyofo et al. (1989) that lactose inhibits Salmonella colonization because it promotes : 1) lactobacilli growth and 2) bacteria that compete for S. Typhimurium colonization or produce volatile fatty acids toxic to S. Typhimurium. The action of lactose against

Salmonella in chickens by producing lactic acid is to be expected in the ceca (Ziprin et al., 1991).

Rosemary has an active material which inhibits the bacteria growth and act in a way or other on the variation in the building of cellular wall of the bacteria which lead to the creation of gabs inside the bacteria cytoplasm to the outside region. This intern has negative effect on the vitality of those bacteria (Cabuk *et al.*, 2003). Rosemary essential oil is used as an antibacterial, antifungal (Oluwatuyi et al., 2004; Fernández, 2005). Rosemary have been used as stimulating effects of digestion and antiparasitic, antibacterial on Escherichia coli and Salmonella typhimurium (Singh et al., 2002; Cabuk et al., 2003; Tabancal et al., 2003) and antifungal (Soliman and Badea 2002). This is attributed to terpene fraction, which is composed of borneole, aneole, pinene and camphor. In conclusion 1% rosemary used as antimicrobial balance in intestinal tract for broiler chicks (Ghalib et al., 2008). Rosemary results are in agreement with that of Bolukbasi and Erhan, (2007), where they pointed out the positive effect in decreasing *E.coli* in intestinal tract in order to improve the animal health and performance. They added that the mechanism of the bacterial inhibition effect of aromatic plant through the resulting interference between the contents of rosemary with cellular membrane for microbial germ which led to a change in the diffusion of potassium ion and hydrogen via viability of microorganism.

Economic efficiency:

As given by economical study in Table (6), the best net revenüe or the economic efficiency either during Normal (23°C) or High (38°C) temperature were recorded by the chicks fed lactose followed by rosemary and then citric acid, respectively. These results indicated that the experimental treatments were more economical than the control diet. This improvement could be due to improving the feed conversion or reducing the amount of feed required to produce one unit of egg mass as a result to reducing or controlling the intestinal pathogenic bacteria. Come to similar result Patterson and Burkholder (2003), Snow et al (2004) and Ghalib et al (2008).

Finally, addition of organic acid (citric acid), prebiotic (lactose) or aromatic plant (rosemary) in broiler diet were efficient in controlling pathogenic bacteria in chickens intestine which could act as antibacterial, bacteriostatic, antiparasitic or immunostimulant against pathogenic bacteria especially E. coli, salmonella, staphylococci and Coccidia, consequently, improving broiler performance, physiological and bacteriological status under normal or heat stress condition. They could be beneficial in controlling immunosuppressed environmental conditions and reducing infection and pollution in poultry farms.

Table (1): Composition and calculated analysis of the experimental diets fed to the experimental birds.

Ingredients	Starter diet (1 to 4 wks) %	Finisher diet (5 to 6 wks) %
Yellow corn	50.30	63.25
Soybean meal 44 %	41.00	29.50
Vegetable oil	04.70	03.75
Lime stone	1.20	01.20
Di-calcium phosphate	2.00	01.50
Salt (NaCl)	0.30	00.30
Vit. & Min. Mixture *	0.30	00.30
DL-Methionine 99%	0.20	00.20
Total	100.00	100.00
Calculated analysis		
Metabolizable energy (Kcal / Kg)	3000.27	3098.08
Crude protein %	22.03	18.00
Crude fiber %	4.15	3.61
Crude fat %	7.23	6.60
Calcium %	1.05	0.90
Available phosphorus %	0.52	0.41
Lysine %	1.33	1.02
Methionine %	0.56	0.51
Met + cystine %	0.92	0.81

Supplied per Kg of diet: Vit. A, 10 000 IU; Vit. D_3, 2 000 IU; Vit. E,10 mg; Vit. K_3,1 mg; Vit. B_1, 1mg: Vit. B_2, 5 mg: Vit. B_6, 1.5 mg; Vit. B_{12}, 10 mcg; Niacin, 30mg; Pantothenic acid, 10mg: Folic acid,1mg; Biotin. 50mcg;Choline,260mg; Copper, 4 mg; Iron, 30mg; Manganese, 60mg; Zinc,50mg; Iodine.1.3mg; Selenium. 0.1mg; Cobalt, 0.1mg;

Table (2): Performance of broilers at 6 weeks of age as affected by experimental treatments.

Items		Normal temperature (23°C)				High temperature (38°C)			
		Control	Citric acid	Lactose	Rosemary	Control	Citric acid	Lactose	Rosemary
Weight gain (g)		1548.3 ^{b} ± 39.5	1685.3 ^{a} ±36.43	1669.4 ^{a} ± 29.11	1673.6 ^{a} ± 33.82	1469.6 ^{c} ± 37.7	1573.4 ^{b} ± 30.5	1581.6 ^{b} ± 38.5	1591.5 ^{b} ± 43.4
Feed consumption (g)		3530.2 ^{b} ± 33.3	3776.0 ^{a} ± 45.5	3690.7 ^{a} ± 38.3	3715.2 ^{a} ± 46.8	3247.5 ^{e} ± 28.3	3430.0 ^{c} ± 34.4	3338.2 ^{d} ± 41.1	3421.7 ^{c} ± 36.8
Feed conversion rate		2.28 ^{a} ± 0.04	2.24 ^{b} ± 0.02	2.21 ^{c} ± 0.02	2.22 ^{bc} ± 0.02	2.21 ^{c} ± 0.05	2.18 ^{d} ± 0.02	2.11 ^{f} ± 0.02	2.15 ^{e} ± 0.02
Mortality rate % at	7 - 35	10 ^{a}	3.33 ^{b}	3.33 ^{b}	3.33 ^{b}	-----	----------	----------	----------
	35 - 42	3.70	0.00	0.00	0.00	18.52 ^{a}	6.90 ^{c}	10.34 ^{b}	3.44 ^{d}
Carcass traits									
Pre-slaughter weight (g)		1555.3	1663.4	1645.9	1690.2	1463.6	1544.5	1567.2	1552.4
Carcass (g)		1057.3	1170.4	1155.4	1185.8	981.0	1075.1	1091.7	1082.2
Carcass %		67.98 ^{c}	70.36 ^{a}	70.20 ^{a}	70.15 ^{a}	67.02 ^{d}	69.60 ^{b}	69.66 ^{b}	69.71 ^{b}
Giblets %		5.04 ^{c}	5.31 ^{a}	5.33 ^{a}	5.34 ^{a}	4.72 ^{d}	5.09 ^{b}	5.10 ^{b}	5.14 ^{b}
Dressing %		73.02 ^{c}	75.67 ^{a}	75.50 ^{a}	75.49 ^{a}	71.74 ^{d}	74.69 ^{b}	74.76 ^{b}	74.85 ^{b}
Lymphoid organs									
Bursa %		0.07 ^{b}	0.14 ^{a}	0.14 ^{a}	0.15 ^{a}	0.09 ^{b}	0.13 ^{a}	0.13 ^{a}	0.13 ^{a}
Thymus %		0.26 ^{b}	0.49 ^{a}	0.46 ^{a}	0.48 ^{a}	0.29 ^{b}	0.45 ^{a}	0.47 ^{a}	0.47 ^{a}

Means on the same row differently superscripted are significantly different ($P<0.05$).

Traits measured as percentage have no associated stander error since they are retrains formed estimates.

Table (3): Some blood biochemical parameters of broilers as affected by experimental treatments at 40 days of age.

Items	Normal temperature (23°C)				High temperature (38°C)			
	Control	Citric acid	Lactose	Rosemary	Control	Citric acid	Lactose	Rosemary
T_3 (ng/dl)	241.2^{a} ±11.41	249.40^{a} ± 10.2	250.5^{a} ± 14.10	248.6^{a} ± 8.4	188.90^{b} ± 10.1	195.5^{b} ± 12.8	196.5^{b} ± 9.19	193.0^{b} ± 7.18
Total protein (ng/dl)	4.36^{d} ± 0.10	4.37^{d} ± 0.10	4.38^{d} ± 0.10	4.48^{c} ± 0.07	4.56^{b} ± 0.12	4.55^{b} ± 0.14	4.56^{b} ± 0.10	4.69^{a} ± 0.10
Albumin (g/dl)	2.49^{d} ± 0.02	2.51^{d} ± 0.02	2.49^{d} ± 0.02	2.55^{c} ± 0.02	2.55^{b} ± 0.03	2.56^{ab} ±0.02	2.57^{ab} ± 0.02	2.60^{a} ± 0.02
Globulin (g/dl)	1.87^{d} ± 0.03	1.86^{d} ± 0.02	1.89^{d} ± 0.02	1.93^{c} ± 0.02	2.01^{b} ± 0.03	1.99^{b} ± 0.02	1.99^{b} ± 0.02	2.09^{a} ± 0.02
A/G ratio	1.33^{a} ± 0.01	1.34^{a} ± 0.01	1.31^{a} ± 0.01	1.32^{a} ± 0.01	1.27^{b} ± 0.02	1.28^{b} ± 0.01	1.29^{b} ± 0.01	1.26^{b} ± 0.01
AST (U/L)	22.24^{a} ±2.42	22.14^{a} ±1.19	22.20^{a} ±1.82	22.40^{a} ±1.57	22.73^{a} ±1.55	22.25^{a} ±1.25	22.46^{a} ±1.08	22.36^{a} ±1.23
ALT (U/L)	43.34^{a} ±3.02	43.05^{a} ±2.33	42.88^{a} ±2.42	43.15^{a} ±2.08	44.20^{a} ±3.63	43.77^{a} ±2.63	43.50^{a} ±2.44	43.34^{a} ±2.29
Creatinine (mg/dl)	0.79^{a} ± 0.02	0.78^{a} ± 0.02	0.78^{a} ± 0.02	0.78^{a} ± 0.02	0.79^{a} ± 0.02	0.78^{a} ± 0.02	0.79^{a} ± 0.02	0.77^{a} ± 0.02
Total lipids (mg/dl)	376.51^{a} ± 6.80	340.18^{d} ± 4.17	323.50^{d} ± 5.25	312.11^{e} ± 4.26	386.16^{a} ± 6.15	359.15^{c} ±3.87	331.14^{d} ±4.33	313.30^{e} ± 3.49
Cholestrol (mg/dl)	174.82^{a} ± 3.35	135.14^{b} ± 3.55	141.34^{b} ± 3.35	138.20^{b} ± 2.67	178.45^{a} ± 2.97	148.15^{b} ± 4.66	144.28^{b} ± 3.12	141.3^{b} ± 3.82
Hemoglobin (g/dl)	12.10^{a} ±0.81	12.24^{a} ±0.44	12.11^{a} ±0.67	12.28^{a} ±0.34	12.61^{a} ±0.31	12.82^{a} ±0.52	12.21^{a} ±0.35	12.18^{a} ±0.52
Hematocrit (%)	31.13^{a} ±0.13	31.21^{a} ±0. 21	31.44^{a} ±0. 25	31.25^{a} ±0. 28	31.71^{a} ±0. 10	31.25^{a} ±0.37	31.62^{a} ±0. 19	31.45^{a} ±0. 35

Means on the same row differently superscripted are significantly different ($P<0.05$).

Table (4): Blood hematological picture of broilers as affected by experimental treatments at 40 days of age.

Items	Normal temperature (23°C)				High temperature (38°C)			
	Control	Citric acid	Lactose	Rosemary	Control	Citric acid	Lactose	Rosemary
Erythrocytes (/ µl)	2.88^{b} ±0.0 4	$2. 89^{b}$ ±0.02	2.88^{b} ±0.02	2.89^{b} ±0.01	3.01^{a} ±0.03	3.00^{a} ±0.03	3.08^{a} ±0.02	3.06^{a} ±0.02
Leukocytes ($x10^3$ /µl)	11.7^{d} ±0.06	11.8^{d} ±0.08	11.8^{d} ±0.07	12.2^{b} ±0.04	12.1^{c} ±0.04	12.1^{c} ±0.02	12.1^{c} ±0.02	12.5^{a} ±0.04
Heterophil %	30.1^{d} ±0.23	30.1^{d} ±0.23	30.2^{d} ±0.07	31.8^{c} ±0.03	34.9^{b} ±0.03	34.7^{b} ±0.01	34.8^{b} ±0.07	39.0^{a} ±0.03
Lymphocytes %	54.3^{a} ±0.46	54.8^{a} ±0.46	54.5^{a} ±0.09	54.0^{a} ±0.15	42.2^{b} ±0.07	42.1^{b} ±0.03	42.0^{b} ±0.09	42.3^{b} ±0.15
Eosinophil %	4.2^{a} ±0.11	4.0^{a} ±0.11	4.2^{a} ±0.01	4.2^{a} ±0.01	3.4^{b} ±0.64	3.5^{b} ±0.05	3.5^{b} ±0.01	3.5^{b} ±0.01
Basophil %	10.3^{a} ±0.06	10.0^{a} ±0.06	10.1^{a} ±0.08	10.0^{a} ±0.01	8.00^{b} ±0.04	8.00^{b} ±0.08	8.5^{b} ±0.08	8.2^{b} ±0.01
Monocyte %	1.01 ±0.00	1.1 ±0.00	1.0 ±0.00	0.0 ±0.00	11.5^{a} ±0.08	11.7^{a} ±0.06	11.2^{a} ±0.00	7.0^{b} ±0.00

Means on the same row differently superscripted are significantly different ($P< 0.05$).

Table (5): Counts of some intestinal pathogenic bacteria and parasite (CFU /g fluid) of broilers as affected by experimental treatments at 40 days of age.

Items	Normal temperature (23°C)				High temperature (38°C)			
	Control	Citric acid	Lactose	Rosemary	Control	Citric acid	Lactose	Rosemary
Ileum								
Aerobic plate count (x10^{6}/g)	7.02±0.6^{b}	3.85±0.3^{h}	5.36±0.2^{e}	4.18±0.4^{g}	8.92±0.7^{a}	5.16±0.3^{f}	6.63±0.5^{c}	5.87±0.4^{d}
E. coli (x10^{6}/g)	6.31±0.4^{d}	5.19±0.2^{f}	5.87±0.3^{e}	4.94±0.2^{g}	8.65±0.6^{a}	6.71±0.4^{c}	7.51±0.5^{b}	6.74±0.3^{c}
Salmonella (x10^{3}/g)	15±0.2	0.00	0.00	0.00	23±0.4	0.2±0.6^{c}	0.00	0.00
Staphylococci (x10^{3}/g)	36±0.2^{b}	27±0.3^{d}	29±0.8^{c}	29±0.6^{c}	39±0.3^{a}	26±0.4^{d}	29±0.5^{c}	21.2±0.2^{e}
Coccidia (x10^{3}/g)	4±0.3	0.00	0.00	0.00	16±0.6	0.00	0.00	0.00
Caecum								
Aerobic plate count (x10^{7}/g)	7.22±0.4^{c}	5.10±0.2^{g}	6.07±0.5^{d}	4.10±0.3^{h}	8.75±0.9^{a}	5.42±0.3^{e}	6.71±0.4^{c}	5.22±0.2^{f}
E. coli (x10^{6}/g)	5.12±0.6^{d}	4.33±0.4^{g}	4.76±0.4^{e}	3.83±0.2^{h}	7.52±0.6^{a}	5.36±0.3^{c}	5.68±0.4^{b}	4.49±0.3^{f}
Salmonella (x10^{3}/g)	14 ±0.3	0.00	0.00	0.00	17 ±0.2	0.00	0.00	0.00
Staphylococci (x10^{3}/g)	35±0.03^{b}	32±0.3^{c}	33±0.5^{c}	31±0.4^{d}	39±0.3^{a}	32±0.2^{c}	34±0.4^{b}	32±0.4^{c}
Coccidia (x10^{3}/g)	13±0.06	0.00	0.00	0.00	21±0.1	0.00	0.2±0.6^{c}	0.00
Count/g fecal matter								
Aerobic plate count (x10^{7}/g)	8.06±0.6^{b}	5.65±0.3^{e}	6.52±0.2^{c}	5.36±0.4^{f}	9.15±1.4^{a}	5.13±0.4^{g}	6.54±0.3^{c}	6.12±0.4^{d}
E. coli (x10^{6}/g)	6.43±0.4^{c}	4.93±0.3^{g}	5.13±0.3^{f}	4.76±0.2^{h}	8.33±0.6^{a}	6.10±0.4^{d}	6.68±0.4^{b}	5.94±0.3^{e}
Salmonella (x10^{3}/g)	14±0.1	0.00	0.00	0.00	16±0.1	0.00	0.00	0.00
Staphylococci (x10^{3}/g)	41.1±0.2^{c}	38.9±0.4^{e}	39.3±0.7^{d}	34.1±0.6^{g}	47.0±0.2^{a}	37.0±0.4^{f}	43±0.5^{b}	3.93±0.2^{d}
Coccidia (x10^{3}/g)	18±0.02	0.00	0.00	0.00	23±0.02	0.00	0.4	0.00

Means on the same row differently superscripted are significantly different ($P<0.05$).

Table (6): Economical efficiency of of broilers at 42 days of age as affected by experimental treatments.

Items	Treatments	Normal temperature (23°C)				High temperature (38°C)			
		Control	Citric acid	Lactose	Rosemary	Control	Citric acid	Lactose	Rosemary
Feed	Total intake (Kg/chick)	3.53	3.77	3.69	3.71	3.24	3.43	3.33	3.42
	Price / kg (L.E)	2.03	2.04	2.04	2.05	2.03	2.04	2.04	2.05
	Cost (L.E)	7.17	7.69	7.52	7.60	6.58	6.99	6.79	7.01
Meat	Wight gain	1.548	1.685	1.669	1.673	1.469	1.573	1.581	1.591
	Price / kg (L.E)	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
	Total Revenue (L.E)	17.71	19.38	19.19	19.24	16.91	18.09	18.18	18.30
Net Revenue (L.E)		10.54	11.69	11.67	11.64	10.33	11.10	11.39	11. 29
Economic efficiency		147.00	151.98	155.18	153.15	156.99	158.79	167.77	161.05
Relative Economic efficiency (%)		100	103.39	105.57	104.18	100	101.15	106.85	102.58

Net revenue = Price of Wight gain / chick - feed cost

Economic efficiency = net revenue / feed cost x 100

Relative economic efficiency (%) assuming the Control treatments = 100%

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الملخص العربي

تخفيض الكائنات الحية الدقيقة المرضية في أمعاء بداري إنتاج اللحم تحت الظروف العادية أو المجهدة

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أجريت هذه الدراسة لمعرفة تأثير بعض المركبات مثل الأحماض العضوية (حامض الستريك) والبريبيوتك أو بادئات البكترية الحيوية (سكر اللاكتوز) والنباتات العطرية (مسحوق أوراق نبات حصي لبان) التي ربما تعمل على تقليل أو التحكم في عدد البكتيريا المعوية التي تسبب الأمراض المعوية التي تسبب خسائر إقتصادية كبيرة في حالة تعرض الطيور لظروف بيئية مجهدة أو ضاغطة وبالتالي تحسين بعض الصفات الإنتاجية والفسيولوجية لكتاكيت اللحم اثناء التربية العادية (٢٣م). وكذلك أثناء الإجهاد الحرارى (٣٨م). تم ستخدام ٢٤٠ كتكوت هبرد (هجين تجارى لانتاج اللحم) وزعت عشوائيا إلى أربع مجموعات متساوية بكل منها ٦٠ كتكوت (٢مكرر) وتم تربيتهم تحت ظروف متماثلة. المعاملة الأولي تم تغذيتها علي عليقة المقارنة (بدون إضافات). و المعاملة الثانية تم تغذيتها علي عليقة المقارنة مع إضافة الحامض العضوي (حامض الستريك) بمعدل ٢جم / كجم عليقه. المعاملة الثالثة تم تغذيتها علي عليقة المقارنة مع إضافة بادئات البكترية الحيوية (لسكر اللاكتوز) بمعدل ٢جم / كجم عليقه. المعاملة الرابعة تم تغذيتها علي عليقة المقارنة مع إضافة النبات العطري (مسحوق أوراق نبات حصي لبان) بمعدل ١%. عند عمر ٣٥ يوم تم تقسيم كل معاملة إلى مجموعتين متساويتين كل منها ٣٠ كتكوت. الأولى تم تربيتها تربية عادي (٢٤م) حتى نهاية التجربة والثانية تم تعريضها لمدة ٤ أيام للإجهاد الحراري (٣٨م) لمدة ٣ ساعات / اليوم من عمر ٣٥ حتى ٤٠ يوم . ويمكن عرض أهم النتائج فيما يلى :-

١. هناك زيادة معنوية (P<0.05) للإضافات التجريبية المستخدمة في علائق كتاكيت إنتاج اللحم تحت ظروف التربية العادية أو التعرضة للإجهاد الحراري على وزن الجسم المكتسب والعلف المأكول مع الكفاءة التحولية ومعدل النفوق. وكذلك زيادة معنوية (P<0.05) على الوزن النسبي لكل من الذبيحة والأجزاء المأكولة وكذلك الغدد اللمفاوية (غدة البرسا وغدة التيموسية).

٢. أدت إضافة مسحوق أوراق حصى لبان إلي زيادة معنوية (P<0.05) للبروتين الكلى وبالمثل مستوى الألبيومين والجلوبيولين في بلازما الدم. كما أدت ايضا إلي إنخفاض معنوي فى كل من مستوى الكولسترول والدهون الكلية في بلازما الدم. بينما لم يتأثر معنوياً كل من تركيز الهيموجلوبين والهيماتوكريت وكذلك مستوى هرمون تراي أيودوثيرونين (T_3) الكرياتين وإنزيمات AST and ALT في بلازما الدم. وكذلك الإضافات التجريبية الأحري لم يكن لها تأثير علي أي من مكونات الدم سواء فى الظروف العادية أو ظروف الإجهاد الحراري.

٣. كما أدت إضافة مسحوق أوراق حصي لبان إلي زيادة معنوية (P<0.05) في كل من خلايا الدم البيضاء Leukocytic (WBC's) وخاصة Heterophil . وعلي العكس إنخفضت معنوياً خلايا Monocyte . بينما لم يتأثر معنوياً كل من خلايا من نوع Lymphocytes Eosinophil and Basophil, وكذلك خلايا الدم الحمراء Erythrocytic (RBC's) . الإضافات التجريبية الأخري لم يكن لها تأثير علي أي من مكونات الدم سواء فى الظروف العادية أو ظروف الإجهاد الحراري.

٤. كما أدت للإضافات التجريبية المستخدمة في العلائق كتاكيت إنتاج اللحم تحت ظروف التربية العادية أو التعرض للإجهاد الحراري على إلي إنخفاض شديد في العدد الكلى للبكتريا المرضية و E. Coli و staphylococci في الزرق أو في الإمعاء (والصائمي والاعور) ولكن بكتيريا Salmonella وطفيل الكوكسيديا Coccidia قد إختفتا تماما في الزرق أو في الإمعاء (والصائمي والاعور) تحت ظروف التربية العادية أو التعرض للإجهاد الحراري.

٥. - التعرض للإجهاد الحراري أدى إلي زيادة معنوية (P<0.05) لمعدل النفوق والعدد الكلى للبكتريا المرضية المختبرة ، بينما انخفض معنويا (P<0.05) كل من وزن الجسم المكتسب والعلف المأكول ووزن الذبيحة. كما أدت إضافات العلفية المستخدمة الي تقليل أوتحسين الأثار السيئة لأرتفاع درجة الحرارة

ويستخلص من النتائج أنة يمكن استخدام حامض الستريك أو سكر اللاكتوز أو مسحوق أوراق نبات حصي لبان في عليقة كتاكيت أنتاج اللحم لتقليل أو التحكم في عدد البكتيريا المعوية التي تسبب الأمراض المعوية لتجنب الخسائر الإقتصادية التي قد تكون كبيرة وكذلك لتحسين معدل الزيادة في وزن الجسم ومعدل التحويل الغذائى والصفات الفسيولوجية وخفض معدل النفوق في الأجواء الحارة أو في الظروف البيئية المجهدة