



South Valley University
Faculty of Veterinary Medicine
Department of Poultry Diseases



STUDYING THE ROLE OF NATURALLY MUTANT NON-PATHOGENIC O27 STRAIN OF *E. COLI* AS A CANDIDATE FOR A PROBIOTIC IN BROILERS

Thesis presented by

Shimaa Abdelrahim Mohammed Ahmed
(B. V. Sc. 2011, M. V. Sc. 2017; South Valley University)

Submitted for
The degree of Ph.D.
(Poultry Diseases)

Under supervision of

Prof. Dr. Ahmed Ibrahim Ahmed

Emeritus Professor of Poultry Diseases
Faculty of Veterinary Medicine
South Valley University

Assistant Prof. Dr. Nabila Mahmoud Mohammed

Assistant professor of Poultry Diseases
Head of Poultry Diseases Department
Faculty of Veterinary Medicine
South Valley University

Dr. Dina Mohamed Waheed Shibat El-hamd

Researcher of Poultry Diseases
Animal Health Research Institute, Qena
Agricultural Research Center

1441H-2020AD

Table of contents

List of Tables	I
List of Figures	II
List of abbreviations	VI
1.Introduction	1
2.Review of literatures	
2.1. Definition of probiotics	4
2.2. History of probiotics	4
2.3. Type of probiotic and micro-organisms used in poultry	5
2.4. Effects of probiotics in poultry	6
2.5. Mechanisms of action	16
2.6. Criteria for selection of probiotics in the poultry industry	19
2.7. Salient benefits, usage recommendation, best time for administration and common forms at which probiotics are available	19
2.8. Probiotics or antibiotics?	21
2.9. <i>E. coli</i> as a probiotic	22
2.10. Naturally mutant non-pathogenic O27 strain of <i>E. coli</i>	24
2.11. Pathogenomics of virulence genes of <i>Escherichia coli</i>	26
3.Materials and Methods	36
3.1. Studying the basic molecular genetic characteristics of O27 strain of <i>E. coli</i>	36
3.2. Studying the age at which the probiotic can be administered, the effect of naturally mutant non-pathogenic O27 strain of <i>E. coli</i> as a probiotic on growth performance, hematological and biochemical	43

parameters, induction of chicken antibody response and histopathological studies	
3.3.1. Studying the antagonistic activities of naturally mutant non-pathogenic O27 strain of <i>E. coli</i> as a probiotic against the infection of pathogenic Enterobacteriaceae <i>in vitro</i>	57
3.3.2. Studying the antagonistic activities of naturally mutant non-pathogenic O27 strain of <i>E. coli</i> as a probiotic against the infection of pathogenic Enterobacteriaceae <i>in vivo</i> and comparing it with another commercial probiotic and commercial antibiotic	59
4.Results	64
4.1. Studying the basic molecular genetic characteristics of O27 strain of <i>E. coli</i>	64
4.2. Studying the pathogenicity, the age at which the probiotic can be administered, the effect of naturally mutant non-pathogenic O27 strain of <i>E. coli</i> as a probiotic on growth performance, hematological and biochemical parameters, induction of chicken antibody response and histopathological studies	66
4.3.1. Studying the antagonistic activities of naturally mutant non-pathogenic O27 strain of <i>E. coli</i> as a probiotic against the infection of pathogenic <i>Enterobacteriaceae</i> <i>in vitro</i>	72
4.3.2. Studying the antagonistic activities of naturally mutant non-pathogenic O27 strain of <i>E. coli</i> as a probiotic against the infection of pathogenic <i>Enterobacteriaceae</i> <i>in vivo</i> and comparing it with another commercial probiotic and commercial antibiotic	73
5.Discussion	86
6. Conclusion	98
7. Summary	100
8. References	104
Arabic summary	

List of tables

Table No.	Title	Page No.
Materials and methods		
3.1	Oligonucleotide primers sequences.	37
3.2	Preparation of PCR Master Mix.	41
3.3	Cycling conditions of the different primers during cPCR.	41
3.4	Types, number in each 100 cells and morphology of WBCs cells in chicken.	48
Results		
4.1	Results of virulence genes of O27 strain by PCR.	64
4.2	Statistical analytical results of the effect of O27 strain supplementation on growth performances of broiler chickens in different ages.	67
4.3	Types and number in each 100 cells of WBCs cells in chicken.	68
4.4	Effects of O27 strain of <i>E. coli</i> supplementation on different blood biochemical parameters in broiler chickens.	69
4.5	Immune response (HI antibody titer) against NDV vaccine and (ELISA antibody titer) to a vaccine strain of IBD virus in broiler chickens as influenced by O27 strain of <i>E.coli</i> supplementation.	70
4.6	Antagonistic activities of O27 strain against salmonella typhimurium and O1 strain of <i>E. coli</i> .	72
4.7	Mortality rate difference between groups	78
4.8	Statistical analytical results of performance parameters and nutrient utilization in different groups.	79
4.9	Types and number in each 100 cells of WBCs cells in chicken	80
4.10	Blood biochemical parameters in different groups in broiler chickens.	81
4.11	Immune response (HI antibody titer) to NDV vaccine and (ELISA antibody titer) to a vaccine strain of IBD virus in broiler chickens in different groups.	82

List of figures

Figure No.	Title	Page No.
Results		
4.1	PCR products of amplified of virulent genes identified in O27 strain of <i>E. coli</i> visualized on agarose gel electrophoresis.	65
4.2	28 day old chicks from the control group (1) on the left and from the group (2) show full vitality and activity.	66
4.3	28 day old chickens from the group (1) on the left, group (2) in the middle and group (3) on the right, all of them showing normal liver and heart.	66
4.4	28 day old chickens from the group (1) on the left, group (2) in the middle and group (3) on the right, all of them show normal deudenum.	67
4.5	28 day old chickens from the group (1) on the left, group (2) in the middle and group (3) on the right, all of them show normal jejunum.	67
4.6	28 day old chickens from the group (1) on the left, group (2) in the middle and group (3) on the right, all of them show normal cecum.	67
4.7	Mean weekly body weight /grams of the 3 groups. Group (2) showed the highest body weight along the experiment.	68
4.8	Weekly feed consumption /grams of the 3 groups. Group (2) showed the highest feed consumption along the experiment.	68
4.9	Performance Index of the 3 groups. Group (2) showed the highest performance along the experiment.	68
4.10	Types and number in each 100 cells of WBCs cells of the 3 groups showing normal hematological parameters with no difference between groups.	69
4.11	Blood biochemical parameters of the 3 groups showing normal biochemical parameters with no difference between groups.	70
4.12	Immune response (HI antibody titer) against NDV vaccine of the 3 groups. G2 showed the highest antibody titer.	71
4.13	Immune response (ELISA antibody titer) against IBD vaccine of the 3 groups. G2 showed the highest antibody titer.	71
4.14	Jejunal sections from 28 day old chicken showing normal villi and normal active glands in both pictures with more intact and densely packed microvilli in the left sample from group (2) than the right sample from group (1).	71
4.15	Deudenal section from 28 day old chicken showing normal villi and normal deudenal gland, all of the 3 groups showing the same picture.	71

4.16	Cecal section from 28 day old chicken showing normal cecal glands and villi, all of the 3 groups showing the same picture.	71
4.17	Showing the antagonistic activities of O27 strain against salmonella typhimurium.	73
4.18	Showing the antagonistic activities of O27 strain against O1 strain of E.coli.	73
4.19	13 day old chicks showing weight difference between the control group on the right side and the group (2) in the right side.	74
4.20	13 day old chicks showing weight difference between the control group on the right side and the group (5) in the right side.	74
4.21	17 day old chick from group (2) showing depression, ruffled feathers, mouth-breathing.	74
4.22	17 day old chick from group (5) showing depression, ruffled feathers and fuel-smelling diarrhea.	74
4.23	35 day old chickens from group (3) on the right side and group (4) on the left side showing full vitality with no clinical signs.	74
4.24	35 day old chickens from group (2) on the right side and group (5) on the left side showing improvement, full vitality with no clinical signs.	74
4.25	30 days old chick showing normal carcass (the same p/m picture in group 1, 3, 4 and 6).	75
4.26	30 days old chick showing normal heart (the same p/m picture in group 1, 3, 4 and 6).	75
4.27	30 days old chick showing normal liver (the same p/m picture in group 1, 3, 4 and 6).	75
4.28	30 days old chick showing normal air sacs in both pictures (the same p/m picture in group 1, 3, 4 and 6).	75
4.29	30 days old chick showing normal kidneys in the left side and normal intestine in the right side (the same p/m picture in group 1, 3, 4 and 6).	76
4.30	17 days old chick infected with O1 strain showing colisepticemia with airsacculitis, pericarditis and perihepatitis (the same p/m picture in groups 2 & 5).	76
4.31	17 days old chick showing kidney affection in the left side and air sacculitis in the right side (the same p/m picture in groups 2 & 5)	76
4.32	28 days old chicken showing different degree of improvement of the whole carcass after administration of O27 strain of <i>E. coli</i> with remnant fibrin on the heart on the right side and complete recovery of the heart on the left side with complete recovery of both livers (the same p/m picture in groups 2 & 5).	77
4.33	28 days old chicken showing improvement of liver with slight haemorrhage on its surface after administration of O27 strain of <i>E. coli</i> (the same p/m picture in groups 2 & 5).	77

4.34	28 days old chicken from group (2 & 5) showing improvement of lung with slight amounts of fibrinous exudates after administration of O27 strain of <i>E. coli</i> in the right side comparing to complete recovery of the lung on the left side from the same groups.	77
4.35	28 days old chicken from group (2 & 5) showing improvement of kidneys with slight enlargement after administration of O27 strain of <i>E. coli</i> in the left side comparing to complete recovery of the kidneys on the right side from the same groups.	77
4.36	Mean weekly body weight /grams of the 6 groups.	80
4.37	Weekly feed consumption /grams of the 6 groups.	80
4.38	Performance Index of the 6 groups.	80
4.39	Types and number in each 100 cells of WBCs cells of the 6 groups showing normal hematological parameters except heterophils decreased in groups (2 & 5)	81
4.40	Blood biochemical parameters of the 6 groups showing normal biochemical parameters with no difference between groups.	82
4.41	Immune response (HI antibody titer) against NDV vaccine of the 6 groups.	83
4.42	Immune response (ELISA antibody titer) against IBD vaccine of the 6 groups.	83
4.43	Jejunum section from 35 day old chicken from group (6) showing normal villi and normal active glands with intact and densely packed microvilli.	83
4.44	Jejunum section from 35 day old chicken from group (3) and the same in group (4) showing abnormal villi with mild mononuclear cell infiltration.	83
4.45	Jejunum section from 35 day old chicken from group (2) and the same in group (5) showing mild necrosis and mild degenerative changes in the villi and inflammation, activation of mucous glands and congestion from group (2) and the same in group (5)	83
4.46	Deudenal section from 35 day old chicken from group (6) and the same in group (1) showing normal tall villi and normal deudenal gland, all of the 3 groups showing the same picture.	84
4.47	Deudenal section from 35 day old chicken from group (3) and the same in group (4) showing nearly normal leaflet villi with moderate activated crypts.	84
4.48	Deudenal section from 35 day old chicken from group (2) and the same in group (5) showing hyperplasia and hyperactivation of goblet cells with few inflammatory cells.	84
4.49	Cecal section from 35 day old chicken from group (6) and the same in group (1) showing normal cecal glands and villi.	84
4.50	Cecal section from 35 day old chicken from group (3) and the same in group (4) showing normal cecal glands and villi, only mild congestion of submucosal blood vessels with few inflammatory cell	84

7. Summary

The purpose of the current study was to evaluate the molecular characteristics of naturally mutant nonpathogenic O27 strain of *E. coli* and its efficacy as probiotic in broilers, determine the best age at which it can be administered and studying its antagonistic activities against the pathogenic bacteria in vitro and in vivo and comparing its effects with another commercial probiotic and antibiotic.

For studying the molecular characteristics of naturally mutant nonpathogenic O27 strain of *E. coli*, 24 virulence genes using 24 sets of primers were detected using PCR technique. The data revealed that *FimH*, *iroN*, and *crl* genes were detected in O27 strain, but *Tsh*, *Hly*, *papC*, *KpsMTII*, *Stx1*, *Stx2*, *ibeA*, *eaeA*, *STa*, *LT*, *astA*, *cvaC*, *VT2e*, *Cnf1*, *CFAI*, *CFAIII*, *CS2*, *CS4*, *Pic*, *Vat*, and *espP* were not detected in O27 strain.

About studying of O27 strain effects as probiotic on performance of broilers, enhancement the immunity and determining the best age at which it can be administered, 65 chicks (1 day old) were arranged into three groups, 20 per group, and reared for four weeks. The remaining 5 chicks were randomly sacrificed and were examined bacteriologically for pathogenic *E. coli*. All the results were *E. coli* negative. First group was negative control, second group was treated orally with O27 strain at 1st day of life for three successive days and repeated at 21st day old chicks, and third group were administered orally with O27 strain at 10th day old and repeated at 21st day old. The results were as the following: Groups (1, 2 and 3) appeared very healthy showing full vitality and activity with no mortalities or postmortem lesions along the experiment. Group (2) performance parameters were significantly better ($p < 0.01$) than groups (3 & 1). Hematological and biochemical parameters did not be influenced ($p > 0.05$) by the administration of O27 strain. Antibody titers of IBDV and NDV in groups (2 & 3) were improved as compared to group (1). Group (2) had significantly higher titers than group (3). Histopathologically, all groups showed normal histopathological pictures, but jejunum in groups (2 & 3) showed taller, intact, and densely packed microvilli and more crypt depth than the control group (1).

Studying the antagonistic activities of O27 strain of *E. coli* as a probiotic against pathogens in vitro was determined by co-cultivation with different pathogenic *Enterobacteriaceae* (For example: *salmonella typhimurium* and O1 strain of *E. coli*) by using paper disc method, the results revealed that at the concentration of (1.5×10^8 cells /ml) from O27 strain of *E. coli*, the widest inhibitory zone was formed against *salmonella typhimurium* (23 mm) and O1 strain of *E. coli* (27 mm) and at the concentrations of (1.5×10^7 cells /ml) and (1.5×10^6 cells /ml) gave an inhibitory zone of (20 mm) and (15 mm) respectively against O1 strain of *E. coli* with no activity against *salmonella typhimurium* at these concentrations.

For studying the antagonistic activities of O27 strain of *E. coli* as a probiotic against the infection of pathogenic *Enterobacteriaceae* in vivo, and comparing it with commercial probiotics and commercial antibiotics, 125 chicks (day 1 old) were arranged into six groups, 20 per group, and reared for five weeks. The remaining 5 chicks were randomly sacrificed and were examined bacteriologically for pathogenic *E. coli*. All the results were *E. coli* negative. Group (1) was negative control. Group (2) was positive control, orally inoculated with O1 strain at the 10th day of life, then administered orally with O27 strain of *E. coli* at 21st day of life. Group (3) were administered orally with O27 strain of *E. coli* for 3 successive days from the 1st day, then orally inoculated with O1 strain at the 10th day, then administered orally with O27 strain of *E. coli* at 21st day of life. Group (4) were administered orally with commercial probiotic from the 1st day, then orally inoculated O1 strain at the 10th day then administered orally with commercial probiotic at 21st day of life. Group (5) were administered orally with commercial antibiotic for 3 successive days from the 1st day, then orally inoculated with O1 strain at the 10th day then administered orally with O27 strain of *E. coli* at 21st day of life. Group (6) were administered orally with O27 strain of *E. coli* for 3 successive days from the 1st day of life then administered orally with O27 strain of *E. coli* at 21st day of life.

Birds in groups (2 & 5) revealed clinical signs from the 3rd day post-infection (at 13th day old) and increased by time. Chicks showed firstly loss of weight then listlessness,

tendency to huddle together, loss of appetite, fuel-smelling diarrhea, depression, ruffled feather, dropping of wings, and finally showed respiratory signs including gasping (mouth breathing), sneezing and rales. After administration of O27 strain of *E. coli* in group (2 & 5) at 21st day old, birds in the 2 groups showed gradual improvement and subsiding of clinical signs and chickens were apparently normal after 3 days of administration. Groups (2 & 5) before administration of O27 strain showed moderate to severe lesions of enteritis, air sacculitis, pericarditis, perhepatitis, congestion, hemorrhage and enlargement of kidneys and congestion in spleen and other paranchymatus organs during postmortem examination. After administration of O27 strain of *E. coli* in the same groups at age of 21st day old, some birds showed different degree of improvement and others showed complete recovery and became normal. Groups (2 & 5) showed (25%) mortalities until they administered O27 strain of *E. coli* at the age of 3 weeks, after that the 2 groups didn't record any mortalities administered O27 strain of *E. coli* at the age of 3 weeks. Groups (1, 3, 4, and 6) appeared very healthy showing full vitality and activity with no mortalities or postmortem lesions along the experiment.

At the first week of age (before infection), all groups showed no significant ($P>0.05$) changes in mean weekly body weight and performance index, but there is significant increase ($P<0.01$) in feed consumption in groups (3, 4 and 6). At the second and third weeks of age (after infection), groups (3, 4 and 6) showed significant increase in mean weekly body weight ($P<0.05$), feed consumption ($P<0.01$) and performance index ($P<0.05$) than group (1), but group (6) showed higher performance parameters compared to those in the other two groups then group (3) then (4), while groups (2 & 5) showed significant decrease in performance parameters than group (1). At the fourth and fifth weeks of age, groups (3, 4 and 6) showed significant increase ($P<0.01$) in performance parameters than group (1), but group (6) showed higher performance parameters compared to those in the other two groups then group (3) then (4). Groups (2 & 5) still showing significant decrease in body weight gain and performance index, comparing to control group (1), but higher than the previous weeks, and interestingly showed significant increase in feed consumption comparing to control group (1).

The haematological parameters were not influenced ($P>0.05$) in all groups, except heterophils decreased significantly ($P<0.01$) with increasing in lymphocytes count in groups (2 & 5). There is a significant increase ($P<0.05$) in globulin in group (3) then group (4) then groups (2 & 5) with no significant ($P>0.05$) difference in other biochemical parameters in the 6 groups.

The birds in group (3) had significantly ($P<0.01$) & ($P<0.05$) higher titers of antibodies against NDV and IBDV respectively than those in group (4) followed by groups (2 & 5) followed by group (6) and finally group (1) showed the lowest levels of titers.

Histopathological finding showed normal histological pictures in groups (1 & 6), slight lesions in organs of groups (3 & 4) and different degrees of lesions referring to recovery from infection in organs of groups (2 & 5).