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EFFECT OF PROBIOTICS AND PREBIOTICS ON SALMONELLA COLONIZATION AND IMMUNITY IN LOCAL BROILERS' PUREBREDS AND CROSSBREDS

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ABSTRACT

To study the effect of Probiotics and lactose on the Salmonella colonization and immunity in Inshas and Matrouh local broilers purebreds and crossbreds, ten groups of broilers chicks were categorized and offered different treatments of probiotics including *Lactobacillus acidophilus, Bacillus subtilis and Enterococcus faeceium* alone or accompanied by 2.5% Lactose in drinking water. Different parameters were evaluated including body weight, feed conversion, feed intake, daily gain, livability, caecal Salmonella count, caecal pH and antibody titer against Salmonella. Results showed that, *Enterococcus faeceium* had significant effects on body weight and daily gain while none of the used treatments had significant effects on livability of the examined chicks. *Enterococcus faeceium* and *Bacillus subtilis* had significant effects on feed intake only at 7 days of age while *Bacillus subtilis* showed a significant difference on feed conversion only at 28 days age. Inshas x Matrouh crossbred proved to be the most effective in reducing Salmonella count at 28 days. All treatments caused reduction of caecal pH and *Lactobacillus acidophilus* with lactose 2.5% had the highest effect. Matrouh x Inshas crossbred showed the strongest immunity reaction against Salmonella if compared with the other breeds. *Enterococcus faeceium* together with lactose gave also the strongest immune reaction against Salmonella if compared with the other breeds.

INTRODUCTION

Transmission of enteric pathogens to the public contacts of farm animals is a growing problem, particularly among children and old people (Smith, *et al.*, 2004). One of the most frequent causative agents of food infections is *Salmonella*, which mostly can be found in animal herds (Fehlhaber, 2003).

Salmonellae are facultative intracellular Gram-negative bacteria that are found ubiquitously in nature and have the ability to infect wide range of hosts including humans, domesticated and wild mammals and birds. The principal clinical manifestations associated with *Salmonella* infection in humans are enteric fever (typhoid and paratyphoid) and a self-limiting gastroenteritis (salmonellosis) (Salez and Malo, 2004).

Some *Salmonella species* are less pathogenic to birds (notably *Salmonella typhimurium* and *Salmonella enteritidis*) and can cause colonization of the gut, which leads to carcass contamination and subsequent human infection, without causing evident disease in the chicken (Bumstead. 2003).

As control of this health hazard, antimicrobials were used as growth promoter and/or prophylactic agents against many pathogens that may enter the animal body through contaminated carcass meals, edible plastics, sewage, petrochemical residues and excrements (El Moghazy, 2002). These antimicrobials include: Bacitracin, Chlortetracycline, Erythromycin,

Lincomycin, Neomycin, Oxytetracycline, Penicillin, Streptomycin, Tylosin and Verginiamycin, which were added as growth promoters in poultry feed at a level of about 1400 g per ton of feed, which is lower than its minimum inhibitory concentration (subtheraputic level) and consequently encourages the selection of antibiotic resistant bacteria.

As a result of the fact that, many of these antimicrobials are identical to or closely resemble drugs used in human treatment, antimicrobial resistance can be transferred to human bacteria causing serious health hazard (McEwen; and Fedorka-Cray, 2002).

As a result of the widespread of these multiresistant strains of pathogenic bacteria, many health hazards can be occurred specially in the field of human health. The expected consequences are: (I) The appearance of infections that would not have otherwise occurred, (II) The increased frequency of treatment failures and (III) The increased severity of infection including longer duration of illness, increased frequency of bloodstream infections, increased hospitalization and increased mortality (Angulo *et al.*, 2004).

Recently, many countries, including Egypt, banned the usage of antimicrobials as growth promoters after the alarm raised by World Health Organization due to the increase in the incidence of antibiotic resistant strains of *Salmonella* and many other pathogens as a result of using of antibiotics in intensive breeding (El Moghazy, 2002).

Alternatives to growth-promoting and prophylactic uses of antimicrobials in agriculture include improved management practices, wider use of vaccines and introduction of probiotics, prebiotics and a combination of them (synbiotic) (McEwen; and Fedorka-Cray, 2002).

Probiotics, which means "for life" in Greek, has been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989).

The mode of action of these types of bacteria as growth promoters can occur via inhibition of the pathogenic bacteria found in the intestinal tract of animals and poultry .Among these bacteria is *Lactobacillus acidophilus*, *Enterococcus faecium* and *Bacillus subtilis* that have strong beneficial effect in reducing the colonization of Salmonella species in market-aged broilers (Guo *et al.*, 1990).

The role of *Enterococcus faecalis* is through its lactic acid and bacteriocin production which create acidic pH in the intestinal tract preventing the colonization of pathogenic bacteria specially Salmonella in the intestine (Carina Audisio *et al.*, 2000).

Lactobacillus strains can be considered as potential ingredients for a chicken probiotic feed formulation intended to control salmonellosis; also, they improve poultry sanitation due to their production of lectins, which have a marked antimicrobial effect (Gusils *et al.*, 1999).

In addition, *Bacillus subtilis* in poultry diets improve live performance of broilers in the absence of antibiotics and may contribute to on-farm pathogen reduction (Fritts *et al.*, 2000).

Prebiotics are defined as "a non digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or

activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995)

Lactose, as a commonly used prebiotic, markedly increases resistance to caecal colonization, organ invasion and horizontal transmission of *Salmonella* species in broilers when included in drinking water. The main role of this prebiotic is achieved through its utilization by the intestinal beneficial bacteria resulting in; reduced caecal pH, increased caecal lactic acid, acetic acid, propionic acid and buteric acid concentration and increased caecal oxidation-reduction potential which in return considerably reduces *Salmonella* colonization in caeca of treated birds (El Borollosy *et al.*, 2001).

The aim of this work is to find safe growth promoters for chickens to be used as alternatives to antimicrobial growth promoters through estimation of the effect of three different probiotic strains (*Lactobacillus acidophilus, Enterococcus faecium and Bacillus subtilis*), Lactose and a mixture of all on:

1- Antibody titer against Salmonella in the serum of artificially inoculated broiler chicks.
2- Count of Salmonella living cells in their caeca.
3- Different performance parameters.

MATERIALS AND METHODS

A- Materials

Chicks:

Four hundred eighty one day old chicks were obtained from Anshas Research Station, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.

The experimental chicks belonged to 4 genetic groups:

a. ♀Matrouh X Matrouh ♂ (120 chicks).
b. ♀Anshas X Anshas ♂ (120chicks).
c. ♀Matrouh X Anshas ♂ (120 chicks).
d. ♀Anshas X Matrouh ♂ (120 chicks).

Water samples:

Ten Samples from the source of water offered to the chicks were collected to be examined for the presence of *Salmonella*.

Feed samples:

Ten Samples from feed offered to the chicks were collected to be examined for the presence of *Salmonella*.

Litter samples:

Samples from the litter present in the floor in which the chicks were delivered were examined for the presence of *Salmonella*.

Cloacal swab samples:

Two hundred chicks (five chicks from every treatment per genetic group) were examined for the presence of *Salmonella* by cloacal swab.

Bacterial strains:

***Salmonella*:**

Salmonella typhimurium was kindly obtained from Animal Health Research Institute, A.R.C., Giza, Egypt.

Probiotic strains:

The used strains of *Lactobacillus acidophilus, Enterococcus faecalis, Bacillus subtilis* were isolated, purified, identified and stored from routine work in the Food Safety Laboratory, Regional Center for Food and Feed, A.R.C., Giza, Egypt.

Experimental diets:

Starter, grower and finisher diets were adequately supplied to cover the requirements according to NRC (1994).

The experimental diets (in mash form), the clean as well as residual feed were weighed. Mortality was recorded daily during the experimental period.

B- Methods:

The preparation of infective dose of *Salmonella*:

Salmonella typhimurium was propagated onto S.S agar medium and incubated at 37°C for 24hours, and the growth was harvested, then washed three times and resuspended in phosphate buffer saline. The suspension was matched with Brown's Opacity tube number (1) in order to have a final concentration of 10^6 microorganisms per ml.

Layout of experiment:

The chicks were housed in the floor with wire border under continuous fluorescent lighting, and were provided unmedicated corn soybean-based meal ration (containing no added antibiotics, coccidiostats, or growth promoters) and water *ad libitum*. The chicks were randomly assigned to four genetic groups in each group ten treatments, 12 chicks per treatment. Group A (♀Matrouh X Matrouh ♂), group B (♀Anshas X Matrouh ♂), group C (♀Anshas X Anshas ♂), and (♀Matrouh X Anshas ♂). The treatments in each genetic group were as follows:

1-Treated with lactose 2.5% in drinking water at the day of hatch.
2- Treated with lactose 2.5% in drinking water at the day of hatch and *Lactobacillus acidophilus* at the 1st day of age.
3- Treated with lactose 2.5% in drinking water at the day of hatch and *Enterococcus faecalis* at the 1st day of age.
4- Treated with lactose 2.5% in drinking water at the day of hatch and *Bacillus subtilis* at the 1st day of age.
5- Treated with lactose 2.5% in drinking water at the day of hatch and *Lactobacillus acidophilus, Enterococcus faecalis,* and *Bacillus subtilis* at the 1st day of age.
6-Control negative group without any treatment.
7-Control positive group treated with *Salmonella typhimurium* only.
8- Treated with *Lactobacillus acidophilus* at the 1st day of age.
9- Treated with *Enterococcus faecalis* at the 1st day of age.
10- Treated with *Bacillus subtilis* at the 1st day of age.

Chicks were challenged with 10^6(*Lactobacillus acidophilus, Enterococcus faecalis,* and *Bacillus subtilis*) by crop inoculation at one day of age.

All chicks were challenged with 10^6 Salmonella typhimurium by crop inoculation at 3days of age except control negative. 2.5% lactose in drinking water till the end of experiment was offered.

Detection of *Salmonella* was carried out according to NMKL (1994)

Biochemical and serological identification of *Salmonella*:

Initial identification attempts were made using the criteria described by NMKL (1994) and API 20E (bioMerieux).

The strips were used according to the detailed procedure steps illustrated in the kit's manual. Serological identification of the suspected *Salmonella* strain was also carried out according to NMKL (1994).

Determination of caecal colonization by *Salmonella typhimurium*:

Caecal material was serially diluted in sterile saline solution and plated on brilliant green agar. The plates incubated for 18-24 hours at 37°C, and cfu were counted. Typical *Salmonella* colonies were confirmed by biochemical tests as mentioned before.

Determination of pH in the caecal contents:

At thirty days of age and at the end of experiment, 5 chicks from each treatment/genetic group were slaughtered by cervical dislocation. Caecal contents were aseptically removed, and 0.2 g was suspended in 0.8 ml of sterile glass distilled water. One ml of distilled water was added to the suspension.

Estimation of Salmonella antibody titer in the serum of experimental chicks:

Collection of serum, procedure and interpretation of the results were performed according (Alton *et al.*, 1988).

Experimental Methodology:

The criteria of response (Performance parameters) are recorded & calculated in the present study according to Abdel-Azeem, (1997) which included: live body weight, live body weight gain, feed intake and feed conversion.

Statistical analysis was performed using Proc mixed model in analysis

RESULTS AND DISCUSSION

Economical traits

Effects of different treatments on body weight were illustrated in (Table 1). Body weight of chicks treated with *Enterococcus faecalis* was the heaviest at 21, 28, 35, 42, 49, 56, 63 and 70 days of age when compared with control group (without any treatment) at the same ages which were 162.46, 224.08, 319.50, 402.71, 490.47, 581.72, 704.45 and 847.53 grams, respectively, while means of body weight for control group were 150.49, 211.68, 244.81, 312.10, 383.31, 453.07, 582.91 and 699.82 grams respectively. These results are in agreement with those reported by other investigators (Shivani-Katoch *et al.*, 1996 and Kahraman *et al.*, 1997). The body weight of chicks treated with *Bacillus subtilis* at 35, 42, 49, 56, 63 and 70 days appeared to follow the above mentioned treatment in its effect with values of 292.70, 367.43, 441.55, 520.28, 649.95 and 789.49 grams, respectively.

Table (1): Least - squares means and standard error of body weight (gm) traits* as affected by treatments in a crossbreeding experiment.

Treatment	BW7	BW14	BW21	BW28	BW35	BW42	BW49	BW56	BW63	BW70
1	66.25^{bc} ±1.50	98.64^{bc} ±2.54	147.52^{bcd} ±4.03	187.38^{de} ±5.40	239.10^{cde} ±11.27	301.38^{cde} ±18.14	368.10^{cd} ±21.56	448.37^{cde} ±25.86	587.63^{cd} ±37.2	717.41^{c} ±42.92
2	67.88^{bc} ±1.45	98.26^{bc} ±2.45	145.26^{bcd} ±3.88	173.44^{f} ±5.20	208.14^{f} ±9.70	255.69^{f} ±14.93	317.62^{e} ±17.74	388.38^{f} ±21.28	524.36^{e} ±32.89	651.13^{d} ±37.85
3	65.19^{cd} ±1.44	100.12^{bc} ±2.44	145.42^{bcd} ±3.88	179.43^{ef} ±5.38	225.45^{def} ±11.20	288.40^{de} ±18.06	330.19^{e} ±21.46	425.20^{def} ±25.75	553.99^{de} ±37.22	680.66^{cd} ±42.83
4	60.56^{d} ±1.50	92.99^{c} ±2.50	140.33^{cd} ±3.97	169.33^{f} ±5.32	215.12^{ef} ±11.05	272.42^{ef} ±17.89	321.04^{e} ±21.26	407.19^{ef} ±25.50	538.03^{cde} ±36.97	654.08^{cd} ±42.55
5	63.92^{cd} ±1.47	98.01^{bc} ±2.47	147.78^{bcd} ±3.93	170.85^{f} ±5.26	221.95^{def} ±11.12	287.45^{cde} ±17.96	333.48^{de} ±21.35	412.32^{ef} ±25.61	537.19^{de} ±37.07	654.67^{cd} ±42.66
6	73.45^{a} ±1.44	111.09^{a} ±2.38	150.49^{bc} ±3.82	211.68^{ab} ±5.11	244.81^{c} ±10.55	312.10^{cd} ±17.32	383.31^{c} ±20.59	453.07^{cd} ±24.70	582.9^{bc} ±36.22	699.82^{c} ±41.69
7	68.22^{bc} ±1.44	100.996^{bc} ±2.42	138.61^{d} ±3.85	198.79^{cd} ±5.16	250.26^{cd} ±10.68	323.52^{cd} ±17.47	384.03^{c} ±20.76	455.98^{cde} ±24.91	581.2^{cd} ±36.48	703.75^{cd} ±41.98
8	72.36^{a} ±1.44	102.21^{b} ±2.38	150.08^{bcd} ±3.77	208.13^{bc} ±5.05	248.14^{cd} ±9.98	323.82^{c} ±17.37	388.90^{c} ±20.64	472.53^{c} ±24.76	601.73^{bcd} ±36.34	707.68^{c} ±42.00
9	70.54^{ab} ±1.48	102.48^{b} ±2.45	162.46^{a} ±3.90	224.08^{a} ±5.28	319.50^{a} ±11.00	402.71^{a} ±17.83	490.47^{a} ±21.19	581.72^{a} ±25.42	704.45^{a} ±36.88	847.53^{a} ±42.45
10	67.54^{bc} ±1.45	100.12^{bc} ±2.41	157.79^{ab} ±3.82	227.73^{a} ±5.17	292.70^{b} ±10.13	367.43^{b} ±17.65	441.55^{b} ±20.97	520.28^{b} ±25.16	649.95^{b} ±36.70	789.49^{b} ±42.23

*BW= Body weight at 7 days and up to 70 days, respectively.

*Treatments as described in materials and methods.

a-f means with the same letters within each column of trait are non-significantly different (P<0.05).

These results are in agreement with those reported by other investigators (Jin *et al.*, 1996 and Samanya and Yamauchi 2002). The results showed that, addition of lactose in drinking water to chicks has negative effects on body weight when compared with control group. On contrary, (Maiorka *et al.*, 2001 and Douglas *et al.*, 2003) found that the addition of 2 or 4% lactose increased weight gain (P< 0.08) from zero to 21 days that may increase growth of commercial broiler chicks which may be due to breed variation

Effects of different treatments on daily gain were illustrated in (Table 2). Daily gain of chicks treated with *Enterococcus faecalis* was higher than others during the intervals 28-56 and 7-70 days of age when compared with all treatments at the same ages. Means of daily gain for *Enterococcus faecalis* group were 12.33 and 12.28 grams, respectively. These results are in agreement with those reported by other investigators (Cho *et al.*, 1992 and Pisarski *et al.*, 1995), then daily gain of chicks treated with *Bacillus subtilis* during the intervals 7-28 and 56-70 days of age. Means of daily gain for *Bacillus subtilis* group were 7.63 and 17.70 grams, respectively. Daily gain of chicks treated with *Lactobacillus acidophilus* has no significant differences when compared with control group (without any treatment) during all intervals of the experiment. The results showed that, addition of lactose in drinking water to chicks has negative effects on daily gain when compared with control group. On contrary, (Maiorka *et al.*, 2001 and Douglas *et al.*, 2003) found that the addition of 2 or 4% lactose increased weight gain (P< 0.05) from zero to 21 days that may increase growth of commercial broiler chicks.

Table (2): Least-squares means and standard error of daily gain (gm) traits[+] as affected by treatments in a crossbreeding experiment.

Treatment*	DG 7d:28d	DG 28d:56d	DG 56d:70d	DG 7d: 70d
1	5.74^{cd} ±0.22	9.32^{bc} ±0.70	17.71^{a} ±1.77	10.31^{c} ±0.66
2	5.02^{e} ±0.22	7.88^{d} ±0.58	17.27^{a} ±1.56	9.26^{d} ±0.58
3	5.43^{de} ±0.22	9.15^{bc} ±0.70	16.72^{a} ±1.77	9.73^{cd} ±0.66
4	5.17^{de} ±0.22	8.81^{bc} ±0.69	16.13^{a} ±1.75	9.40^{cd} ±0.65
5	5.06^{e} ±0.22	8.64^{c} ±0.69	15.80^{a} ±1.76	9.33^{cd} ±0.66
6	6.59^{b} ±0.21	8.87^{bc} ±0.67	16.14^{a} ±1.72	9.93^{c} ±0.64
7	6.19^{bc} ±0.21	9.09^{c} ±0.67	16.16^{a} ±1.73	10.00^{cd} ±0.64
8	6.47^{b} ±0.21	9.56^{bc} ±0.67	15.59^{a} ±1.73	10.06^{cd} ±0.64
9	7.33^{a} ±0.22	12.33^{a} ±0.69	17.49^{a} ±1.75	12.28^{a} ±0.65
10	7.63^{a} ±0.21	10.41^{b} ±0.68	17.70^{a} ±1.74	11.41^{b} ±0.65

[+] DG=daily gain at 7:28, 28:56, 56:70 and 7:70 days of age.

* Treatments as described in Table 1.

[a-c] means with the same letters within each column of trait are non-significantly different (P<0.05).

There were no significant differences among different treatments on livability (Table 3). These results are in agreement with those reported by other investigators (Senani *et al.*, 1997 and Shivani-Katoch *et al.*, 2000), who found that probiotic and prebiotic effect did not significantly affect the livability at different ages. However, Kahraman *et al.*, 1997 and Jin *et al.*, 2000 reported that probiotic and prebiotic have significant effects on livability trait.

Table (3): Least-squares means and standard error of livability traits * as affected by treatments in a crossbreeding experiment.

Treatment*	L1	L2	L3	L4	L10
1	0.939 [a] ±0.04	0.911 [a] ±0.05	0.912 [a] ±0.05	0.911 [a] ±0.05	0.998 [a] ±0.015
2	0.976 [a] ±0.04	0.950 [a] ±0.04	0.950 [a] ±0.04	0.950 [a] ±0.05	0.999 [a] ±0.014
3	0.975 [a] ±0.04	0.950 [a] ±0.04	0.950 [a] ±0.04	0.913 [a] ±0.05	0.999 [a] ±0.015
4	0.936 [a] ±0.04	0.922 [a] ±0.04	0.923 [a] ±0.05	0.922 [a] ±0.05	1.000 [a] ±0.015
5	0.961 [a] ±0.04	0.935 [a] ±0.04	0.936 [a] ±0.05	0.935 [a] ±0.05	1.001 [a] ±0.015
6	0.983 [a] ±0.04	0.981 [a] ±0.05	0.965 [a] ±0.05	0.964 [a] ±0.05	0.999 [a] ±0.015
7	0.972 [a] ±0.04	0.944 [a] ±0.04	0.944 [a] ±0.04	0.944 [a] ±0.04	1.000 [a] ±0.013
8	0.971 [a] ±0.04	0.971 [a] ±0.04	0.971 [a] ±0.04	0.971 [a] ±0.04	0.955 [a] ±0.013
9	0.946 [a] ±0.04	0.946 [a] ±0.04	0.946 [a] ±0.05	0.932 [a] ±0.05	1.000 [a] ±0.015
10	0.961 [a] ±0.04	0.960 [a] ±0.04	0.960 [a] ±0.04	0.946 [a] ±0.04	1.000 [a] ±0.014

* L =Livability at 1st, 2nd, 3rd, 4th and 10th week of age, respectively.
* Treatments as described in Table 1.
[a-c] means with the same letters within each column of trait are non-significantly different (P<0.05).

There were not significant differences among different treatments on feed intake except at 7 days of age, the highest feed intake for group which was treated with *Enterococcus faecalis* and *Bacillus subtilis* group then *Lactobacillus acidophilus* (Table 4).

There were no significant differences among different treatments on feed conversion except at 28 days of age the highest feed conversion for group which treated with *Bacillus subtilis* and 2.5% lactose group then *Enterococcus faecalis* and 2.5% lactose group then *Lactobacillus acidophilus* and 2.5% lactose group (Table 5). At 42 days of age the highest feed conversion for group which treated with *Lactobacillus acidophilus* and 2.5% lactose then *Enterococcus faecalis* and 2.5% lactose group and *Bacillus subtilis* and 2.5% lactose group.

Table (4): Least – squares means and standard error of feed intake traits* as affected by treatments in a crossbreeding experiment.

Treatment	BW7	BW14	BW21	BW28	BW35	BW42	BW49	BW56	BW63	BW70
1	66.25^{bc} ±1.50	98.64^{bc} ±2.54	147.52^{bcd} ±4.03	187.38^{cde} ±5.40	239.10^{cde} ±11.27	301.38^{cde} ±18.14	368.10^{cd} ±21.56	448.37^{cde} ±25.86	587.63^{cd} ±37.2	717.41^{c} ±42.92
2	67.88^{bc} ±1.45	98.26^{bc} ±2.45	145.26^{bcd} ±3.88	173.44^{f} ±5.20	208.14^{f} ±9.70	255.69^{f} ±14.93	317.62^{e} ±17.74	388.38^{f} ±21.28	524.36^{e} ±32.89	651.13^{d} ±37.85
3	65.19^{cd} ±1.44	100.12^{bc} ±2.44	145.42^{bcd} ±3.88	179.43^{ef} ±5.38	225.45^{def} ±11.20	288.40^{cde} ±18.06	330.19^{de} ±21.46	425.20^{def} ±25.75	553.99^{cde} ±37.22	680.66^{cd} ±42.83
4	60.56^{cd} ±1.50	92.99^{c} ±2.50	140.33^{cd} ±3.97	169.33^{f} ±5.32	215.12^{ef} ±11.05	272.42^{ef} ±17.89	321.04^{e} ±21.26	407.19^{ef} ±25.50	538.03^{cde} ±36.97	654.08^{cd} ±42.55
5	63.92^{cd} ±1.47	98.01^{bc} ±2.47	147.78^{bcd} ±3.93	170.85^{f} ±5.26	221.95^{def} ±11.12	287.45^{cde} ±17.96	333.48^{de} ±21.35	412.32^{ef} ±25.61	537.19^{de} ±37.07	654.67^{cd} ±42.66
6	73.45^{a} ±1.44	111.09^{a} ±2.38	150.49^{bc} ±3.82	211.68^{ab} ±5.11	244.81^{c} ±10.55	312.10^{cd} ±17.32	383.31^{c} ±20.59	453.07^{cd} ±24.70	582.9^{bc} ±36.22	699.82^{c} ±41.69
7	68.22^{bc} ±1.44	100.996^{bc} ±2.42	138.61^{d} ±3.85	198.79^{cd} ±5.16	250.26^{cd} ±10.68	323.52^{cd} ±17.47	384.03^{c} ±20.76	455.98^{cde} ±24.91	581.2^{cd} ±36.48	703.75^{cd} ±41.98
8	72.36^{a} ±1.44	102.21^{b} ±2.38	150.08^{bcd} ±3.77	208.13^{bc} ±5.05	248.14^{cd} ±9.98	323.82^{c} ±17.37	388.90^{c} ±20.64	472.53^{c} ±24.76	601.73^{bcd} ±36.34	707.68^{c} ±42.00
9	70.54^{ab} ±1.48	102.48^{b} ±2.45	162.46^{a} ±3.90	224.08^{a} ±5.28	319.50^{a} ±11.00	402.71^{a} ±17.83	490.47^{a} ±21.19	581.72^{a} ±25.42	704.45^{a} ±36.88	847.53^{a} ±42.45
10	67.54^{bc} ±1.45	100.12^{bc} ±2.41	157.79^{ab} ±3.82	227.73^{a} ±5.17	292.70^{b} ±10.13	367.43^{b} ±17.65	441.55^{b} ±20.97	520.28^{b} ±25.16	649.95^{b} ±36.70	789.49^{b} ±42.23

* FI =feed intake at 1st to 10th week of age..

* Treatments as described in Table 1.

a-e means with the same letters within each column of trait are non-significantly different (P<0.05).

Table (5): Least-squares means and standard error of feed conversion traits * as affected by treatments in a crossbreeding experiment.

Treatment*	FC1	FC2	FC3	FC4	FC5	FC6	FC7	FC8	FC9	FC10
1	0.11 ^{ab} ±0.11	0.29 ^{b} ±0.05	0.46 ^{a} ±0.16	0.61 ^{bcd} ± 0.45	1.28 ^{b} ±1.76	0.56 ^{abc} ±0.12	0.56 ^{bcd} ± 0.19	0.54 ^{a} ± 0.21	0.33 ^{a} ±0.13	0.50 ^{ab} ± 0.08
2	0.12 ^{ab} ±0.06	0.36 ^{ab} ±0.03	0.38 ^{a} ±0.09	0.99 ^{bc} ±0.26	4.17 ^{a} ± 0.98	0.74 ^{a} ±0.07	0.81 ^{bc} ± 0.11	0.68 ^{a} ±0.11	0.52 ^{ab} ±0.07	0.63 ^{a} ± 0.04
3	0.14 ^{ab} ±0.05	0.36 ^{ab} ±0.03	0.43 ^{a} ±0.08	1.49 ^{ab} ±0.24	1.93 ^{ab} ± 0.88	0.64 ^{ab} ±0.06	1.31 ^{a} ± 0.10	0.46 ^{a} ±0.11	0.49 ^{ab} ±0.07	0.54 ^{ab} ± 0.04
4	0.15 ^{a} ±0.06	0.39 ^{a} ±0.03	0.35 ^{a} ±0.09	1.86 ^{a} ±0.26	1.11 ^{b} ±0.98	0.61 ^{ab} ±0.07	0.84 ^{bc} ±0.12	0.51 ^{a} ±0.13	0.43 ^{b} ±0.08	0.57 ^{a} ±0.05
5	0.00 ^{b} ±0.06	0.31 ^{b} ±0.03	0.31 ^{a} ±0.09	1.23 ^{abc} ±0.23	0.87 ^{b} ±0.88	0.50 ^{abc} ±0.06	0.88 ^{b} ± 0.10	0.60 ^{a} ±0.11	0.46 ^{ab} ±0.07	0.52 ^{ab} ±0.04
6	0.08 ^{ab} ±0.06	0.26 ^{b} ±0.03	0.48 ^{a} ±0.09	0.53 ^{d} ±0.25	1.70 ^{ab} ±0.98	0.49 ^{bc} ±0.07	0.55 ^{cd} ± 0.11	0.56 ^{a} ±0.12	0.41 ^{b} ±0.07	0.56 ^{ab} ±0.05
7	0.08 ^{ab} ±0.06	0.36 ^{ab} ±0.03	0.45 ^{a} ±0.09	0.64 ^{cd} ±0.25	1.50 ^{b} ±0.95	0.53 ^{abc} ±0.07	0.76 ^{bcd} ± 0.11	0.84 ^{a} ±0.12	0.64 ^{ab} ±0.07	0.56 ^{ab} ±0.05
8	0.10 ^{ab} ±0.06	0.34 ^{ab} ±0.03	0.49 ^{a} ±0.09	0.52 ^{cd} ±0.24	2.22 ^{ab} ±0.92	0.51 ^{bc} ±0.06	0.62 ^{cd} ± 0.11	0.54 ^{a} ±0.12	0.43 ^{b} ±0.07	0.57 ^{ab} ±0.04
9	0.14 ^{ab} ±0.06	0.33 ^{ab} ±0.03	0.35 ^{a} ±0.09	0.45 ^{cd} ±0.25	0.67 ^{b} ±0.94	0 39 ^{c} ±0.07	0.52 ^{d} ± 0.11	0.59 ^{a} ±0.12	0.49 ^{ab} ±0.07	0.45 ^{b} ±0.05
10	0.14 ^{ab} ±0.06	0.32 ^{b} ±0.03	0.34 ^{a} ±0.09	0.50 ^{d} ±0.24	2.09 ^{b} ±1.00	0.51 ^{abc} ±0.07	0.58 ^{cd} ± 0.11	0.64 ^{a} ±0.12	0.49 ^{ab} ±0.07	0.54 ^{ab} ±0.05

* FC =feed conversion at 1st to 10th week of age.

* Treatments as described in Table 1.

a-c means with the same letters within each column of trait are non-significantly different (P<0.05).

Microbiological and Immunological traits

Salmonella colonization and caecal pH:

Tables (6 & 7) revealed that, breed group was found to have highly significant effects (P<0.001) on Salmonella colonization at 28 days of age, while no significant effect of breed on caecal pH was noticed. These results are in agreement with (Girard santosuosso *et al.*, 1998 and Kaiser and Lamont, 2001) who reported significant effect of genetic line (P < 0.05) on Salmonella in caecal content. No significant differences between Matrouh purebred and Inshas purebred on salmonella count at 28 days of age. (Inshas x Matrouh) crossbred significantly decreased Salmonella colonization at 28 days of age than (Matrouh x Inshas) crossbred, while no significant differences between (Matrouh x Inshas) crossbred and Matrouh and Inshas purebred on Salmonella colonization at 28 days of age was noticed. However, (Inshas x Matrouh) crossbred significantly decreased Salmonella colonization at 28 days of age than Matrouh and Inshas purebred.

Table (6): *Salmonella* count as affected by breed, and Treatment at 28 days of age.

Group\ Treatment	Matrouh x Matrouh	Inshas x Inshas	Matrouh x Inshas	Inshas x Matrouh
1	10^3	10^3	10^3	negative
2	negative	negative	negative	negative
3	negative	negative	negative	negative
4	negative	negative	negative	negative
5	negative	negative	negative	negative
6	negative	negative	negative	negative
7	10^4	10^4	10^4	10^4
8	negative	negative	negative	negative
9	negative	negative	negative	negative
10	negative	negative	negative	negative

Treatments as described in Table 1.

Also, all the used treatments significantly decreased caecal pH (P<0.001) at 28 days of age, except 2.5% lactose alone in drinking water, while 2.5% lactose and *Lactobacillus acidophilus* recorded the best effect for caecal pH reduction. This result could be attributed to the effect of both *Lactobacillus acidophilus* and lactose which caused the increase of the lactic acid concentrations of their caecal contents, which were directly correlated to decrease caecal pH values Hinton *et al.*, (1990). These results are in agreement with (Hinton *et al.*, 1990; and Vandevoorde *et al.*, 1991) who stated that the addition of probiotic and prebiotic had significant effect on caecal pH, while Kahraman *et al.*, (1997) showed that caecal pH did not differ in group which treated with probiotic from the control group.

Table (7): Caecal pH as affected by breed, and Treatment at 28 days of age.

Group\ Treatment	Matrouh x Matrouh	Inshas x Inshas	Matrouh x Inshas	Inshas x Matrouh
1	7.16	7.17	7.07	6.89
2	6.03	6.07	6.07	5.99
3	6.41	6.73	6.54	6.68
4	6.80	6.70	6.55	6.70
5	6.52	6.73	6.72	6.73
6	7.51	7.60	7.41	7.56
7	7.24	7.49	7.67	7.34
8	6.85	6.58	6.23	6.59
9	6.31	6.67	6.95	6.81
10	6.48	6.67	6.74	7.03

Treatments as described in Table 1.

Antibody titer:

Data from Tables (8, 9 &10) concluded that, breed was found to have highly significant effects ($P<0.01$) on antibody titer at 28 days of age. These results are in agreement with (Girard Santosuosso *et al.*, 1998 and Kaiser and Lamont, 2001) who reported significant effect of genetic line ($P < 0.05$) on immunity against Salmonella in caecal content.

Table (8): Antibody titer as affected by breed, and Treatment.

Group\ Treatment	Matrouh x Matrouh	Inshas x Inshas	Matrouh x Inshas	Inshas x Matrouh
1	1\640	1\640	1\640	1\2560
2	1\640	1\640	1\640	1\640
3	1\640	1\640	1\640	1\640
4	1\640	1\640	1\640	1\640
5	1\640	1\640	1\1280	1\640
6	0	0	0	0
7	1\640	1\640	1\640	1\640
8	1\640	1\640	1\640	1\640
9	1\640	1\1280	1\2560	1\640
10	1\1280	1\1280	1\1280	1\640

Treatments as described in Table 1.

Table (9): Least -squares means and standard error for antibody titer traits as affected by genetic group in purebreds and crossbreds chicks.

Group	Antibody titer
Matrouh x Matrouh	$657.99^{b} \pm 49.65$
Inshas x Inshas	$641.13^{b} \pm 46.63$
Matrouh x Inshas	$862.54^{a} \pm 47.91$
Inshas x Matrouh	$763.57^{ab} \pm 50.10$

a-c Means with the same letters within each column of trait are not-significantly different (P<0.05).

Table (10): Least-squares means and standard-error for Salmonella count and caecal pH traits as affected by treatment in purebreds and crossbreds chicks.

Treatment	Antibody titer
1	$1091.70^{ab} \pm 79.49$
2	$624.01^{c} \pm 79.55$
3	$639.58^{c} \pm 79.49$
4	$647.43^{c} \pm 77.60$
5	$768.29^{c} \pm 77.66$
6	$0.00^{d} \pm 71.38$
7	$641.40^{c} \pm 75.89$
8	$638.71^{c} \pm 72.78$
9	$1288.20^{a} \pm 77.66$
10	$975.32^{b} \pm 75.89$

Treatments as described in Table 1.
[a-c] means with the same letters within each column of trait are non-significantly different (P<0.05).

No significant differences between Matrouh purebred and Inshas purebred on immunity against Salmonella at 28 days of age was noticed. No significant differences between (Matrouh x Inshas) crossbred and (Inshas x Matrouh) crossbred on immunity against Salmonella at 28 days of age, while (Matrouh x Inshas) crossbred had significant differences with Matrouh and Inshas purebreds on immunity against Salmonella at 28 days of age.

Little information has been reported for effects of Probiotic and Prebiotic on chicks' immunity. Treatments were found to have highly significant effects (P<0.001) on immunity against Salmonella at 28 days of age There were significant differences among different treatments on immunity against Salmonella at 28 days of age, the highest antibody titer for group which treated with *Enterococcus faecalis*. 2.5% lactose group appeared to follow the above mentioned treatment in its effect on immunity against Salmonella at 28 days of age.

Bacillus subtilis appeared to follow the above mentioned treatment in its effect on immunity against Salmonella at 28 days of age. However, *Lactobacillus acidophilus* group which treated with or without lactose had no significant effect on antibody titer at 28 days of age when compared with control positive group (treated with Salmonella). *Enterococcus faecalis* group and *Bacillus subtilis* group which were treated with lactose had no significant effect on antibody titer at 28 days of age. Also, the group treated with *Enterococcus faecalis*, *Bacillus subtilis*, *Lactobacillus acidophilus* and 2.5% lactose had no significant effect on antibody titer at 28 days of age when compared with control positive group (treated with Salmonella).

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دراسة تأثير البروبيوتك والبريبيوتك على تحوصل السالمونيلا فى المعى الأعـــور وعلى المناعه فى بعض السلالات المحليه والمخلوطه لدجاج التسمين

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تمت دراسة تأثير البروبيوتك والبريبيوتك على تحوصل السالمونيلا في المعى الأعور وعلى المناعة فى سلالات انشاص و مطروح المحلية النقيه و المخلوطه.

قسمت الكتاكيت إلى ١٠ مجموعات وأعطيت بكتيريا اللاكتوباسيلس أسيدوفيلس والباسيلس سبتيلس والإنتيروكوكاس فيسيوم منفردة أو مع سكر اللاكتوز المضاف إلى مياه الشرب بنسبة ٢,٥%. تم تقييم بعض القياسات مثل الوزن الكلى ومعدل التحويل الغذائى ومعدل إستهلاك الغذاء ومعدل الإستفادة اليومية ومعدل النفوق ودرجة تحوصل السالمونيلا فى المعى الأعور وقيمة الأس الهيدروجينى وكذلك مستوى الأجسام المضادة بالدم .

أثبتت النتائج أنه كان هناك تأثير معنوى لبكتيريا الإنتيروكوكاس فيسيوم على الوزن الكلى ومعدل الإستفادة اليومية مع عدم وجود أى تأثيرات معنوية على معدل النفوق .

كما أثبتت النتائج أنه كان هناك تأثير إيجابى لبكتيريا الإنتيروكوكاس فيسيوم على معدل الإستهلاك اليومى فقط عند ٧ أيام كما كان لبكتيريا الباسيلس سبتيليس تأثير معنوى على معدل التحويل الغذائى عند ٢٨ يوما.

أظهرت النتائج أن جميع المعاملات سببت إنخفاضا معنويا فى الأس الهيدروجينى خاصة بكتيريا اللاكتوباسيلس أسيدوفيلس مجتمعة مع سكر اللاكتوز المضاف إلى مياه الشرب بنسبة ٢,٥% التى أظهرت اقوى تأثير.

سجلت السلالة مطروح X انشاص أكبر رد فعل فى المناعة وكذلك أثبتت النتائج ان بكتيريا الإنتيروكوكاس فيسيوم مجتمعة مع سكر اللاكتوز المضاف إلى مياه الشرب بنسبة ٢,٥% صاحبت أعلى قيمة لمستوى الأجسام المضادة للسالمونيلا بالدم.

وعلى هذا يعتبر إستخدام أنواع البكتيريا المذكورة كبروبيوتيك وكذلك سكر اللاكتوز كبريبيوتك من أفضل البدائل الآمنة التى يمكن إستخدامها كمنشطات للنمو فى دجاج التسمين وكذلك يمكن الإعتماد على الصفة الوراثية الموجودة فى السلالة مطروح X انشاص للحد من إنتشار السالمونيلا أثناء تربية دجاج التسمين.