Effect of probiotics and prebiotics on economic, microbial and immunological traits in local broilers' purebreds and crossbreds chickens

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Abstract

This experiment was carried out to study the effect of probiotics and lactose on growth performance, Salmonella colonization and immunity in Matrouh (MA) and Inshas (IN) local broilers and their crosses. Four hundred and eighty chicks produced from four genetic groups (MA x MA, IN x IN, MA x IN and IN x MA) were used. Ten groups of broilers chicks of each genetic group 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, daily gain, feed intake, feed conversion, caecal Salmonella count, caecal pH and antibody titre against Salmonella. Results showed that *Enterococcus faeceium* had significant effects on body weight and daily gain of chicks. *Enterococcus faeceium* and *Bacillus subtilis* had significant effects on feed intake only at one week of age while *Bacillus subtilis* showed a significant difference on feed conversion only at 4 weeks of age. IN x MA crossbred proved to be the most effective in reducing Salmonella count at 4 weeks of age. All treatments caused reduction of caecal pH and *Lactobacillus acidophilus* with lactose 2.5% had the highest effect. MA x IN crossbred showed the strongest immunity reaction against Salmonella when compared with other breeds. *Enterococcus faeceium* together with lactose gave also the strongest immune reaction against Salmonella when compared with the other breeds.

Keywords: Food safety, chickens, Probiotics, prebiotics, Salmonella control, immunity, purebreds, crossbreds.

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). Salmonella 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 (Gihan El-Moghazy, 2002). These antimicrobials include: Bacitracin, Chlortetracycline, Erythromycin, Lincomycin, Neomycin, Oxytetracycline, Pencillin, 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. 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 (symbiotic) (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). 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 mixture of all on: growth performance, antibody titer against Salmonella in the serum of artificially inoculated broiler chicks and count of Salmonella living cells in their caeca.

Materials and methods

Chicks used

This experiment was carried out in the Poultry Farm of chickens, Faculty of Agriculture at Moshtohor, Benha University, Egypt, in April 2005.

Experimental work

Two local strains of Matrouh (MA) and Inshas (IN) were used. Pullets of each strain were randomly divided into two groups (100 hens / group); the first group was mated with 10 cocks from the same strain, while the second group was mated with 10 cocks from the other strain. Consequently, the pedigreed eggs from each individual breeding pen for the four mating groups (Table 1) were collected daily for ten days and incubated in one hatch then after.

Table 1. Number of chicks used in the experimental work and description of genetic group of sires and dams produced from them

Genetic group* of chicks	No. of chicks	Genetic group of sire	Genetic group of dam
MA x MA	120	MA	MA
IN x IN	120	IN	IN
MA x IN	120	MA	IN
IN x MA	120	IN	MA
Total	480		

* MA and IN= Matrouh and Inshas strains, respectively.

On hatching day, numbers of 120 chicks (12 chicks from each sire) were randomly chosen from each genetic group, then after wing banded to save its genetic groups and immediately transferred to the Moshtohors' Poultry Farm of chickens. Chicks from each genetic group were distributed randomly on ten treatments (12 chicks in each). Description of treatments supplied to the chicks is summarized in Table 2.

Table 2. Description of treatments used in the experimental work.

Treatment No.	Description of treatment
1	2.5% lactose added in drinking water
2	2.5% lactose in drinking water and *Lactobacillus acidophilus*
3	2.5% lactose in drinking water and *Enterococcus faecalis*
4	2.5% lactose in drinking water and *Bacillus subtilis*
5	2.5% lactose in drinking water and *Lactobacillus acidophilus, Enterococcus faecalis,* and *Bacillus subtilis*
6	Control negative group without any treatment
7	Control positive group treated with *Salmonella typhimurium* only
8	Treated with *Lactobacillus acidophilus*
9	Treated with *Enterococcus faecalis*
10	Treated with *Bacillus subtilis*

At hatch, chicks were challenged with 10^6 cfu (*Lactobacillus acidophilus, Enterococcus faecalis,* and *Bacillus subtilis*) by crop inoculation. At 3 days of age, all chicks were challenged with 106 cfu *Salmonella typhimurium* by crop inoculation, except the control negative group. Chicks were reared in floor brooder up to end of the experiment under continuous lighting program (fluorescent lamps, 10 watt/m^2). 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. They were fed (without antibiotics, coccidiostats, or growth promoters) during rearing and growing periods on diet containing 23.01 %, 20 % crude protein, 3.6 %, 3.19 % crude fiber, respectively, as well as *ad libitum* drinking water. All birds were subjected to similar hygienic and environmental conditions and vaccinated against Newcastle and Gambaro diseases.

Procedure of experiment

Examination of Salmonella in materials

Ten samples from the source of water and feed offered to the chicks were collected to be examined for the presence of Salmonella. Samples from the litter present in the floor in which the chicks were delivered were examined also for the presence of Salmonella. In addition, two hundred chicks (five chicks from every treatment per genetic group) were

examined for the presence of Salmonella by cloacal swab.

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

Probiotic strains
The used strain 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.

The preparation of infective dose of Salmonella
Salmonella typhimurium was propagated onto S.S agar medium and incubated at 37^{o}C for 24 hours, 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^{8} microorganisms per ml.

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 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^{o}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).

Data and traits studied
Data of 480 chicks were recorded for traits of body weight (g) at 1st (BW1), 2nd (BW2), 3rd (BW3), 4th (BW4), 5th (BW5), 6th (BW6), 7th (BW7), 8th (BW8), 9th (BW9) and 10th (BW10) weeks of age. Daily weight gains during the periods from 1 to 4 (DG1-4), 4 to 8 (DG4-8), 8 to 10 (DG8-10) and 1 to 10 (DG1-10) weeks of age were computed. Feed intakes were recorded at the intervals of 1 (FI1), 2 (FI2), 3 (FI3), 4 (FI4), 5 (FI5), 6 (FI6), 7 (FI7), 8 (FI8), 9 (FI9) and 10 (FI10) weeks of age and expressed as g/bird/day. Feed conversion values (g feed/g gain) were computed at the intervals of 1 (FC1), 2 (FC2), 3 (FC3), 4 (FC4), 5 (FC5), 6 (FC6), 7 (FC7), 8 (FC8), 9 (FC9) and 10 (FC10) weeks of age.
Salmonella count and caecal pH traits were also studied, as well as antibody titer in serum was estimated according to procedure of (Alton et al., 1988). The antibody titer was expressed as ($-\log_2$). The criteria of response (performance parameters) are recorded & calculated in the present study according to Abdel-Azeem, (1997) which included: live body weight gain, feed intake and feed conversion.

Statistical analysis
Data of body weight, daily gain and feed conversion traits were analyzed using (Model 1), but Salmonella count and caecal pH traits were analyzed using (Model 2); and feed intake and antibody titer traits were analyzed using (Model 3) according to SAS program (SAS, 2004):

$Y_{ijkl} = \mu + G_i + T_j + X_k + (GT)_{ij} + e_{ijkl}$
(Model 1)
$Y_{ijk} = \mu + G_i + T_j + (GT)_{ij} + e_{ijk}$
(Model 2)
$Y_{ijk} = \mu + G_i + T_j + e_{ijk}$
(Model 3)
Where:
Y_{ijkl} and Y_{ijk} = the observation recorded on chick;
μ = the overall mean;
G_i = fixed effect of the ith genetic group;
T_j = fixed effect of the jth treatment;
X_k = fixed effect of kth sex (levels= 1, 2 and 3 for males, females and dead chicks before sexing, respectively);
$(GT)_{ij}$ = Fixed effect of interaction between the ith genetic group and jth treatment; and
e_{ijkl} and e_{ijk}= the random deviation particular to the chick, assumed to be independently randomly distributed with zero mean and variance (σ_e^2).

Duncan's multiple range test (Duncan 1955) was used to detect the significant differences between means of genetic groups.

Results and discussion

Economical traits

Effects of different treatments on body weight were illustrated in (Table 3). Body weight of chicks treated with *Enterococcus faecalis* was the heaviest at 3, 4, 5, 6, 7, 8, 9 and 10 weeks of age when compared with control group (without any treatment) at the same ages which were 102.48, 224.08, 162.46, 319.5, 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, 312.1, 383.31, 453.07, 582.9 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 5, 6, 7, 8, 9 and 10 weeks appeared to follow the above mentioned treatment in its effect with values of 292.7, 367.43, 441.55, 520.28, 649.95 and 789.49 grams, respectively. These results are in agreement with those reported by many 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 the control group. On the contrary, (Maiorka *et al.*, 2001 and Douglas *et al.*, 2003) found that the addition of 2 or 4% lactose increased weight gain ($P<0.01$) from zero up to 21 days that may increase growth of commercial broiler chicks which may be due to breed variation.

Table 3. Least-square means and standard errors of body weight (g) traits[+] as affected by treatments in a crossbreeding experiment.

Treatment[*]	BW1	BW2	BW3	BW4	BW5	BW6	BW7	BW8	BW9	BW10
1	66.25^{bc}	98.64^{bc}	147.52^{bcd}	187.38^{de}	239.10^{cde}	301.38^{cde}	368.10^{cd}	448.37^{cde}	587.63^{cd}	717.41^{c}
	±1.50	±2.54	±4.03	±5.40	±11.27	±18.14	±21.56	±25.86	±37.2	±42.92
2	67.88^{bc}	98.26^{bc}	145.26^{bcd}	173.44^{f}	208.14^{f}	255.69^{f}	317.62^{e}	388.38^{f}	524.36^{e}	651.13^{d}
	±1.45	±2.45	±3.88	±5.20	±9.70	±14.93	±17.74	±21.28	±32.89	±37.85
3	65.19^{cd}	100.12^{bc}	145.42^{bcd}	179.43^{ef}	225.45^{def}	288.40^{de}	330.19^{e}	425.20^{def}	553.99^{de}	680.66^{cd}
	±1.44	±2.44	±3.88	±5.38	±11.20	±18.06	±21.46	±25.75	±37.22	±42.83
4	60.56^{d}	92.99^{c}	140.33^{cd}	169.33^{f}	215.12^{ef}	272.42^{ef}	321.04^{e}	407.19^{ef}	538.03^{cde}	654.08^{cd}
	±1.50	±2.50	±3.97	±5.32	±11.05	±17.89	±21.26	±25.50	±36.97	±42.55
5	63.92^{cd}	98.01^{bc}	147.78^{bcd}	170.85^{f}	221.95^{def}	287.45^{cde}	333.48^{de}	412.32^{ef}	537.19^{de}	654.67^{cd}
	±1.47	±2.47	±3.93	±5.26	±11.12	±17.96	±21.35	±25.61	±37.07	±42.66
6	73.45^{a}	111.09^{a}	150.49^{bc}	211.68^{ab}	244.81^{c}	312.10^{cd}	383.31^{c}	453.07^{cd}	582.9^{bc}	699.82^{c}
	±1.44	±2.38	±3.82	±5.11	±10.55	±17.32	±20.59	±24.70	±36.22	±41.69
7	68.22^{bc}	100.996^{bc}	138.61^{d}	198.79^{cd}	250.26^{cd}	323.52^{cd}	384.03^{c}	455.98^{cde}	581.2^{cd}	703.75^{cd}
	±1.44	±2.42	±3.85	±5.16	±10.68	±17.47	±20.76	±24.91	±36.48	±41.98
8	72.36^{a}	102.21^{b}	150.08^{bcd}	208.13^{bc}	248.14^{cd}	323.82^{c}	388.90^{c}	472.53^{c}	601.73^{bcd}	707.68^{c}
	±1.44	±2.38	±3.77	±5.05	±9.98	±17.37	±20.64	±24.76	±36.34	±42.00
9	70.54^{ab}	102.48^{b}	162.46^{a}	224.08^{a}	319.50^{a}	402.71^{a}	490.47^{a}	581.72^{a}	704.45^{a}	847.53^{a}
	±1.48	±2.45	±3.90	±5.28	±11.00	±17.83	±21.19	±25.42	±36.88	±42.45
10	67.54^{bc}	100.12^{bc}	157.79^{ab}	227.73^{a}	292.70^{b}	367.43^{b}	441.55^{b}	520.28^{b}	649.95^{b}	789.49^{b}
	±1.45	±2.41	±3.82	±5.17	±10.13	±17.65	±20.97	±25.16	±36.70	±42.23

+BW= Body weight at 1 week and up to 10 weeks, respectively.

[*] Treatments as described in Table 2.

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

Results in (Table 4) illustrated that daily gain of chicks treated with *Enterococcus faecalis* was higher than others during the intervals 4-8 and 1-10 weeks 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). Means of daily gain for *Bacillus subtilis* group during the intervals 1-4 and 8-10 weeks of age were 7.63 and 17.70 grams, respectively. Daily gain of chicks treated with *Lactobacillus acidophilus* has no significant differences when compared with the control group (without any treatment) during all intervals of the experiment. The data cleared that, addition of lactose in drinking water to chicks has negative effects on daily gain when compared with control group. On the contrary, (Maiorka *et al.*, 2001 and Douglas *et al.*, 2003) found that addition of 2 or 4% lactose increased weight gain ($P<0.05$) from zero up to 21 days that may increase growth of commercial broiler chicks.

There were not significant differences among different treatments on feed intake except at one week of age (Table 5). The highest feed intake for group which treated with *Enterococcus faecalis* and *Bacillus subtilis* group then *Lactobacillus acidophilus*.

Highly significant differences among different treatments on feed intake except at the 2nd, 6th and 7th weeks of age (Table 5). The highest feed intake was found in group treated with 2.5% lactose at 2, 5, 6 and 7 weeks followed by those treated with

lactobacillus acidophilus and 2.5% lactose 3, 5 and 6 weeks of age. While, the lowest feed intake were found in control groups at all periods of estimation except at the 3rd and 4th weeks of age only. Highly significant effects were found on feed conversion due to treatments applied at all periods of estimation, except at 3 and 8 weeks of age only. The highest feed conversion was found in group treated with Enterococcus faecalis at 4, 5, 6, 7 and 10 weeks of age followed by those treated with control negative group at 6, 7 and 9 weeks of age.

Table 4. Least-squares means and standard errors of daily gain (g) traits[+] as affected by treatments in a crossbreeding experiment.

Treatment*	DG1-4	DG4-8	DG8-10	DG1-10
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 gains during 1-4, 4-8, 8-10 and 1-10 weeks 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).

Table 5. Least-squares means and standard errors of feed intake (g/bird/day) traits[+] as affected by treatments in a crossbreeding experiment.

Treatment*	FI1	FI2	FI3	FI4	FI5	FI6	FI7	FI8	FI9	FI10
1	3.1^{b} ± 0.28	10.83^{a} ±0.43	18.59^{a} ±0.82	27.68^{ab} ±5.40	29.5^{ab} ±1.25	38.32^{a} ±1.62	43.11^{a} ±2.29	47.26^{ab} ±1.98	57.60^{ab} ±3.29	66.39^{ab} ±3.85
2	3.17^{b} ± 0.28	10.84^{a} ±0.43	15.86^{ab} ±0.82	25.69 ±5.20	28.94^{ab} ±1.25	35.75^{a} ±1.62	43.04^{a} ±2.29	51.45^{a} ±1.98	62.46^{a} ±3.29	69.25^{a} ±3.85
3	2.73^{b} ± 0.28	10.80^{a} ±0.43	16.86^{ab} ±0.82	27.88^{ab} ±5.38	27.42^{b} ±1.25	37.28^{a} ±1.62	42.89^{a} ±2.29	47.69^{ab} ±1.98	56.48^{ab} ±3.29	65.73^{ab} ±3.85
4	2.86^{b} ± 0.28	10.93^{a} ±0.43	17.16^{ab} ±0.82	27.13^{ab} ±5.32	30.12^{ab} ±1.25	38.46^{a} ±1.62	44.10^{a} ±2.29	48.12^{ab} ±1.98	56.42^{ab} ±3.29	61.46^{ab} ±3.85
5	3.33^{ab} ± 0.28	11.37^{a} ±0.43	16.87^{ab} ±0.82	26.15^{b} ±5.26	30.14^{ab} ±1.25	39.76^{a} ±1.62	45.40^{a} ±2.29	48.40^{ab} ±1.98	56.34^{ab} ±3.29	64.32^{ab} ±3.85
6	2.95^{b} ± 0.28	10.01^{a} ±0.43	16.21^{ab} ±0.82	26.84^{ab} ±5.11	26.97^{b} ±1.25	35.14^{a} ±1.62	40.56^{a} ±2.29	44.39^{b} ±1.98	50.00^{b} ±3.29	55.49^{b} ±3.85
7	2.95^{b} ± 0.28	10.01^{a} ±0.43	16.21^{ab} ±0.82	26.84^{a} ±5.11	26.97^{a} ±1.25	35.14^{a} ±1.62	40.56^{a} ±2.29	44.39^{ab} ±1.98	50.00^{ab} ±3.29	55.49^{ab} ±3.85
8	3.59^{ab} ± 0.28	10.60^{a} ±0.43	16.80^{ab} ±0.82	26.03^{b} ±5.05	27.96^{b} ±1.25	37.07^{a} ±1.62	41.43^{a} ±2.29	46.87^{ab} ±1.98	52.88^{ab} ±3.29	61.62^{ab} ±3.85
9	4.16^{a} ± 0.28	11.22^{a} ±0.43	17.23^{ab} ±0.82	26.13^{b} ±5.28	28.33^{b} ±1.25	36.25^{a} ±1.62	43.92^{a} ±2.29	48.42^{ab} ±1.98	56.38^{ab} ±3.29	64.03^{ab} ±3.85
10	4.09^{a} ± 0.28	10.71^{a} ±0.43	15.62^{b} ±0.82	26.43^{b} ±5.17	27.82^{b} ±1.25	36.39^{a} ±1.62	44.18^{a} ±2.29	48.86^{ab} ±1.98	55.90^{ab} ±3.29	62.82^{ab} ±3.85

[+] FI = Feed Intake 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).

Table 6. Least-squares means and standard errors of feed conversion (g feed / g gain) traits [+] as affected by treatments in a crossbreeding experiment.

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

[+] FC = Feed conversion at 1st to 10th week of age.

[*] Treatments as described in Table 2.

[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 (7 & 8) revealed that, genetic group was found to have highly significant effects (P<0.001) on Salmonella colonization at 4 weeks of age, while no significant effect of these groups 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 MA purebred and IN purebred on salmonella count at 4 weeks of age. Genetic group of IN x MA crossbred significantly decreased Salmonella colonization at 4 weeks of age than MA x IN crossbred, while no significant differences between MA x IN crossbred and both of MA and IN purebreds on Salmonella colonization at 28 days of age was noticed. However, IN x MA crossbred significantly decreased Salmonella colonization at 4 weeks of age than both of MA and IN purebreds. Also, all the used treatments significantly decreased caecal pH (P<0.001) at 4 weeks 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 (Table 8). 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 . 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. *Salmonella* count as affected by genetic group[+] and treatment at 4 weeks of age.

Treatment[*]	MA x MA	IN x IN	MA x IN	IN x MA
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 2. [+] Genetic groups as described in Table 1.

Table 8. Caecal pH as affected by genetic group[+] and treatment at 4 weeks of age:

Treatment[*]	MA x MA	IN x IN	MA x IN	IN x MA
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 2.
[+] Genetic groups as described in Table 1.

Antibody titer:

Data from Tables (9, 10 and 11) concluded that, genetic group was found to have highly significant effects (P<0.01) on antibody titer at 4 weeks of age, (Table 10). 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. No significant differences between MA and IN purebreds on immunity against Salmonella at 4 weeks of age were noticed (Table 10). No significant differences between MA x IN and IN x MA crossbreds on immunity against Salmonella at 4 weeks of age, while MA x IN crossbred had significant differences with both MA and IN purebreds on immunity against Salmonella at 28 days of age. Little information has been reported for effects of Probiotic and Prebiotic on chicks' immunity.

Table 9. Antibody titer as affected by genetic group[+] and treatment effects.

Treatment[*]	MA x MA	IN x IN	MA x IN	IN x MA
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 2.
[+] Genetic groups as described in Table 1.

Treatment was found to have highly significant effects (P<0.001) on immunity against Salmonella at 4 weeks of age (Table 11). There were significant differences among different treatments on immunity against Salmonella at 4 weeks of age, the highest antibody titer (1288.20) 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 4 weeks of age.

Table 10. Least-squares means and standard errors for antibody titer trait as affected by genetic group of purebreds and crossbreds chicks.

Genetic group[*] of chicks	*Antibody titer*
Matrouh x Matrouh	657.99^{b}
Inshas x Inshas	641.13^{b}
Matrouh x Inshas	862.54^{a}
Inshas x Matrouh	763.57^{ab}

[a-c] Means with the same letters within each column of trait are not-significantly different (P<0.05).
[*] Genetic groups as described in Table 1.

Table 11. Least-squares means and standard errors for antibody titer trait 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 2.
[a-c] means with the same letters within each column of trait are non-significantly different (P<0.05).

Bacillus subtilis appeared to follow the above mentioned treatment in its effect on immunity against Salmonella at 4 weeks of age (Table 11). However, *Lactobacillus acidophilus* group, *Enterococcus faecalis* group and *Bacillus subtilis* group which treated with lactose which treated with or without lactose had no significant effect on antibody titer at 4 weeks of age when compared with control positive group (treated with Salmonella). Also, the group which treated with *Enterococcus faecalis*, *Bacillus subtilis*, *Lactobacillus acidophilus* and 2.5% lactose had no significant effect on antibody titer at 4 weeks of age when compared with control positive group (treated with Salmonella).

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