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EFFECTS OF MONOSPECIES AND MULTISPECIES PROBIOTICS ON PRODUCTIVE PERFORMANCE, INTESTINAL HISTOMORPHOLOGICAL PARAMETERS AND IMMUNE RESPONSE IN BROILERS

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SUMMARY

An experiment was carried out to test the hypothesis that supplementation of broilers with the probiotics; Lactobacillus acidophilus (Lactolife-Av), Lactobacillus acidophilus plus Pichia anomala yeast (Lactolife-Av+) or Lactobacillus acidophilus plus Pichia anomala plus Bacteriophage (Lactolife-Av+B), in water, has beneficial effects on productive performance, intestinal histomorphology and immune response. Six hundred one-day-old male Arbor Acres Plus broiler chicks were assigned at random to four experimental groups, 6 replicates for each. Birds of group 1 received Lactolife-Av, birds of group 2 received Lactolife-Av+, and birds of group 3 received Lactolife-Av+B, in a dosage of 10^4 CFU/bird in drinking water at the 1^{st} and the 10^{th} days of age. Chickens of group 4 (control group) received plain water without treatment. The obtained results indicated that productive performance of the probiotic treated groups was improved their during the entire experimental period (35 days), as compared to the control group. These results were manifested through; significant increase in final body weight (ranging from 64-85 g/bird), decreased feed intake (ranging from 55-105 g/bird), significant improvement in final feed conversion ratio (ranging from 8-15 points), and significant decrease in total mortality rates (2.67 – 4.67 %). At the end of experimental period, the three treated groups had similar production numbers (from 299 to 310.6), with significant superiority over the control birds (263.4). However, No significant effects due to supplementation with probiotics were observed on carcass characteristics.

Also, there were significant increases in intestinal length and diameter, in treated birds. The ileum histomorphology revealed that Villus height and ratio of villus height/crypt depth were significantly greater in all probiotic treated groups than in the control birds. No significant differences were found in crypt depth among the 4 groups. However, the significantly highest villus height and villus height/crypt depth ratio were those of the Lactolife-Av+ treated birds. Moreover, the HI titers against ND vaccine, at 4 and 5 weeks of age, showed significant increase in the probiotics groups as compared to the control birds.

It can be concluded that the three probiotics used in the present study are capable of improving broiler performance and stimulating the immune system, and could be used as natural and safe growth promoters.

Keywords: Probiotics, broilers, productive performance, intestinal morphology, immune response

INTRODUCTION

Recently, increasing concern about antibiotic-related problems (risk of resistant pathogens and antibiotic residues in animal products) resulted in restriction of the use of antibiotics, as growth promoters, in the animal production industry (Houshmand *et al*., 2011). Therefore, to find natural and safe alternatives to antibiotics, different studies have been conducted. In many parts of the world, feed additives, such as probiotics, are being experimented to alleviate the problems associated with the withdrawal of antibiotics from feed. According to the currently adopted definition by FAO/WHO, probiotics are: "live microorganisms (non-pathogenic bacteria and/or yeast) which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). More precisely, a probiotic is defined as “a live microorganisms of nonpathogenic and nontoxic in nature, which when administered through the digestive route, it beneficially affects the host animal by improving its intestinal balance, and be favorable to the host’s health (Fuller, 2001). Probiotics may contain only one, or several (a consortium) different microorganism species. It was reported that multispecies probiotics are more effective than monospecies probiotics (Timmerman *et al*., 2004).

A large number of reports of research using probiotics in poultry have shown very variable results, from almost negative and absent effects to dramatic positive effects. Several researchers have shown positive effects of adding probiotics on broiler performance in terms of body weight, feed intake, feed conversion ratio and mortality rates (Koc *et al*., 2010), carcass characteristics (Kannan *et al*., 2005), immune responses (Khaksefidi and Ghoorchi, 2006 and Karimi *et al*., 2010) and villus height and crypt depth (Samanya and Yamauchi, 2002 and Zhang *et al*., 2005). Lutful (2009) stated that, since probiotics do not result in the development and spread of microbial resistance, they offer immense potential to become an alternative to antibiotics. He concluded that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of growth, modulation of intestinal micro flora and pathogen inhibition, immune modulation, and promoting meat quality of poultry. The ability of probiotic to stimulate the immune system is an additional reason for supporting their use as alternatives to antibiotics for improving animal health and protection against infectious agents (O'Dea *et al*., 2006 and Higgins *et al*., 2007).

On the other hand, no significant effects of probiotics were reported on broiler body weight, feed conversion, mortality rate (Mutus *et al*., 2006; O'Dea *et al*., 2006 and Houshmand *et al*., 2011), carcass characteristics (Seyyedmousa, 2011), immune responses (Balevi *et al*., 2001; Talebi *et al*., 2008 and Taheri *et al*., 2010) or villus height and crypt depth (Houshmand *et al*., 2011 and Luquetti *et al*., 2012).

Over the past several years many researchers have been looking at the potential of bacteriophage as an alternative to antibiotics to prevent and treat poultry diseases, and reduce food borne pathogens. Bacteriophages are viruses that infect and kill bacteria (Huff *et al*., 2005). Bacteriophages are nature's own way of controlling bacteria, and they are safe, because they have no known effects on animal or plant cells. Therefore, it would appear possible to use bacteriophage to prevent and treat bacterial diseases of animals and humans. Several studies have been conducted to show varying effects of treatment of *Salmonella Enteritidis* infection in poultry using different strains of bacteriophages, with outcomes ranging from poor to average (Fiorentin *et al*., 2005, Toro *et al*., 2005 and Hurley *et al*., 2008). In poultry, bacteriophage treatment, either by aerosol spray or drinking water, may be a plausible alternative to antibiotics for the reduction of pathogen infection such as: *Salmonella* (Higgins *et al*., 2005, 2008 and Borie *et al*., 2008) and *E.coli* (Huff *et al*., 2003 and 2006). Huff *et al*. (2010) reported that bacteriophage therapy significantly reduced the mortality rate in the birds challenged with *E.coli*. There is a shortage of information about the effect of bacteriophage treatment on the broiler productive performance.

The objectives of this study were to evaluate the efficacy of three different types of commercially available probiotics approved for use in broiler chickens. It was hypothesized that each of the probiotic treatments would result in improved productive performance, improved intestinal histomorphological parameters, and modulated immune response compared with the broilers not administered with the probiotics.

MATERIAL AND METHODS

The Probiotics

Three probiotics containing dried *Lactobacillus acidophilus*, avian strain, produced by kanzymedipharm, Canada were used. Their trade names were: Lactolife-Av (monospecies Probiotics), Lactolife-Av+ (multispecies Probiotics) and Lactolife-Av+B (multispecies Probiotics plus Bacteriophage). Each of them contained:

1-Lactolife-Av: Lyophilized cake containing $\geq 1x10^8$ CFU/g *Lactobacillus acidophilus*, BatchNo. LL1170.

2-Lactolife-Av+: Lyophilized cake containing $\geq 1x10^8$ CFU/g *Lactobacillus acidophilus* + *Pichia anomola* yeast, containing $\geq 1x10^7$ CFU/g, Batch No. LA1172.

3-Lactolife-Av+B: Lyophilized cake containing $\geq 1x10^8$ CFU/g *Lactobacillus acidophilus* + *Pichia anomala* containing $\geq 1x10^7$ CFU/g + Bacteriophage containing $\geq 1x10^6$ PFU/g, Batch No. LB1177.

Experimental Design

This experiment was carried out according to the national regulations on animal welfare. Six hundred one-day-old male Arbor Acres Plus broiler chicks were assigned at random to four experimental groups. Each treatment consisted of 6 replicates of deep litter pens (2*1 m) with 25 birds per replicate. Birds of group 1 received Lactolife-Av, birds of group 2 received Lactolife-Av+, birds of group 3 received Lactolife-Av+B, in a dosage according to the manufacturer's recommendations (10^4 CFU/bird) in drinking water at the 1st and the 10th day of age. Chickens of group 4 received plain water without treatment.

General Management

The composition of the diets and their calculated analysis are shown in (Table 1). The commercial diets used were formulated to meet the nutrient requirements of the broiler chicks during starter, grower and finisher periods according to the National Research Council (NRC, 1994). Broilers were fed, *ad libitum*, a corn-soybean meal commercial starter diet (23% crude protein and 3000 k cal ME/kg diet) during the first 2 weeks of age, a commercial grower diet (21% crude protein and 3100 kcal ME/kg diet) from 2-4 weeks of age, and a commercial finisher diet (19% crude protein and 3200 kcal ME/kg diet) from 4-5 weeks of age. Semduramicin was added to ration at a concentration of 25 ppm as a coccidiostat. No antibiotics were administrated in water or feed, for the whole experimental period. Birds had free access to water.

The temperature was set at 32^{o}C on the first day, gradually reduced to 24^{o}C by the end of the third week, and until the end of the experiment. The lighting pattern was 23h L: 1h D. All experimented birds were vaccinated against different diseases according to the vaccination programs adopted in most Egyptian chicken broiler farms. They were vaccinated against Newcastle disease (ND) and infectious bronchitis (IB), using Hitchner B_1+ H_{120} live vaccines, at the 7th day of age, and against Avian Influenza (AI) at the 10th day of age, using inactivated H_5N_2 vaccine. Vaccination against infectious bursal disease (IBD) using 228-E strain and revaccination against ND using La Sota strain were given at the 14th and the 18th day of age, respectively. Avian Influenza vaccine was given subcutaneously (in the back of the neck), while all the other vaccines were administered via drinking water. All chickens were kept in environmentally controlled rooms (semi closed system).

Table 1. Composition of the 3-phase diets (g/kg as fed) used and their calculated analysis

Ingredients	Starter	Grower	Finisher
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Soya oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix*	3	3	3
Calculated analysis:			
Crude protein %	23.0	21.0	19.0
Metabolizable energy (kcal/kg)	3000	3100	3200
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Soya oil	30	43.8	40

*Each gram of premix contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron (FeSO4 H2O), 40 mg; copper (CuSO4 5H2O), 7 mg; iodine [Ca(IO3)2], 0.7 mg; selenium (Na2SeO3), 0.3 mg

Measured Parameters:

I. Productive Performance and Carcass Characteristics:

Chicken performance response variables were determined according to North (1984); weekly individually body weight (wt.) and wt. gain were measured on all birds. Weekly feed consumption (g/d/bird), feed conversion ratio (FCR) (g feed/g live body wt. gain) and mortality rate were measured for each replicate. Dead birds were weighed to include their weights in the feed conversion estimation. An index of productivity is the so-called production number, which equals (Kilograms of growth per day * (100 - mortality %) / Feed conversion ratio) * 100 (Timmerman *et al*., 2006) was estimated for each replicate, at the end of the experimental period. Carcass characteristics (dressing %, front part %, hind part % breast meat %, thigh meat %, carcass meat %, and giblets (liver + heart + gizzard) %) were measured at 5 weeks of age, on randomly 5 birds from each replicate.

II. Intestinal Length and Diameter:

Intestine length (duodenum+ jejunum + ileum) and diameter (in the middle of ileum) were measured on 5 birds from each replicate, on the 35th day of age.

III. Ileal Mucosa:

At the end of the experiment, two birds from each replicate were selected at random and sacrificed. Their small intestine were collected and immediately immersed in 10% buffered formalin. After fixation, 2 cm samples were taken from the middle of ileum. The intestinal segments were obtained according to Samanya and Yamauchi (2002). The ileum was considered from the Meckel's diverticulum to ileocecal junction. Routine histological laboratory methods including dehydration, clearing and paraffin embedding were used and paraffin blocks were made, according to Zhang *et al*. (2005). The slides were stained with hematoxylineosin. Histological indices were measured using digital photography and light microscopy. The villous height (μm) was measured from the apical to the basal region, which corresponded to the superior portion of the crypts. Crypts depth (μm) was measured from the base until the region of transition between the crypt and the villus. Three measurements per slide were made for each parameter and averaged into one value.

IV. Immune Response:

For determination of the effect of the probiotics on humoral immunity; blood samples were collected from wing vein of 20 birds, chosen at random, from each group (5 birds/replicate), at weekly intervals (1-5 weeks of age). Serum samples were subjected to HI test for determining antibody titers against ND vaccine employing 8 HA units as described by Swayne *et al*. (1998).

Statistical Analyses:

One-way analysis of variance has been adopted using SAS software general liner models procedure (SAS Institute, 1999). The main factor was probiotic supplementation. Percentage data were subjected to arc sine transformation prior to analysis. Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) when significant differences existed. Significance was set at P<0.05.

RESULTS

I. Productive performance and carcass characteristics:

Results of the administration of Lactolife-Av, Lactolife-Av+ or Lactolife-Av+B in the drinking water of broilers on body weight and body weight gain are presented in Table 2. The results revealed significant increases in final body weight, at 5 weeks of age, for the birds receiving any of the probiotics treatments over the control group. The differences between the probiotics treatments were not significant, at that age. The data of body weight gain (Table 2) indicated no significant differences between the four groups, except from zero to the 1st and from the 2nd to the 3rd weeks of age. At the first interval, the Lactolife-Av+ group had significantly the lowest body weight gain. The control birds had the lowest body weight gain between the 2nd and the 3rd weeks of age.

Results of total feed consumption, per bird, indicated a significant decrease in the probiotic + Bacteriophage treated group than the control group (Table 3). However, there were no significant differences between all the probiotic treated groups. In general, the control group showed less body weight and higher feed intake than the 3 studied probiotic treated groups. Final feed conversion ratio (FCR), for the whole 5 weeks, indicated significant improvements of all treated groups over the control group (Table 3). This improvement in FCR started to be present from the 3rd week of age.

In the present investigation; using the probiotics in the drinking water resulted in significant decreases in the total mortality rates (Table 4). The differences in the weekly mortality rates between the four experimental groups were not statistically significant, throughout the experimental period, except at the 2nd week of age. The control group had significantly higher mortality rate than the other 3 probiotic treated groups, at 2 weeks of age.

As for the production numbers, the results (Table 4) showed that the three probiotic treated groups had similar values (from 299 to 310.6), with significant superiority over the control group (263.4). Data of the present study (Table 5) showed no significant beneficial or detrimental effects of probiotics administration on carcass characteristics.

Table 2. Effects of water supplementation with probiotics on body weight and body weight gain in broilers

Treatment	Body Weight (g)					
	One Day old	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks
Lactolife-Av	39.5± 0.29	132.2± 1.40 ^{a*}	390.7± 3.83	810.2± 6.70^{a}	1302.5± 10.89^{a}	1862.5± 12.57^{a}
Lactolife-Av+	39.9± 0.26	125.5± 1.45^{b}	382.8± 4.81	784.2± 7.70^{b}	1284.4± 11.48^{ab}	1880.0± 13.70^{a}
Lactolife-Av+B	39.7± 0.26	129.5 ±1.41^{a}	395.6± 3.62	793.9± 5.41^{ab}	1267.2± 10.73^{bc}	1858.2± 12.55^{a}
Control	39.9 ± 0.24	132.7± 1.20^{a}	392.5± 3.83	774.6± 6.53^{b}	1249.5± 12.93^{c}	1794.2± 16.85^{b}
Probability	0.7318	0.0006	0.1410	0.0014	0.0091	0.0001
Treatment	**Weekly Body Weight gain (g)**					
	0-1 Week		**1-2 Weeks**	**2-3 Weeks**	**3-4 Weeks**	**4-5 Weeks**
Lactolife-Av	92.6± 1.34^{a}		258.5± 4.09	420.4± 7.91^{a}	492.3± 12.06	531.8± 16.11
Lactolife-Av+	85.6± 1.49^{b}		257.3± 4.92	401.7 ± 9.01^{ab}	499.9± 13.93	563.7± 17.19
Lactolife-Av+B	89.8± 1.4^{a}		266.1± 3.70	398.3± 6.59^{ab}	473.2± 11.15	562.8± 14.44
Control	92.8± 1.26^{a}		259.9± 3.89	383.1± 7.54^{b}	474.4± 14.59	509.2± 20.12
Probability	0.0005		0.4499	0.0100	0.3741	0.0700

* Means with different superscripts, within trait and age, are significantly different (P ≤ 0.05).

-Lactolife-Av = *Lactobacillus acidophilus*, Lactolife-Av+ = *Lactobacillus acidophilus+ Pichia anomola,* and Lactolife-Av+B = *Lactobacillus acidophilus* + *Pichia anomala* +Bacteriophage.

II. Intestinal length and diameter:

Significant increases in intestinal length and diameter for all probiotic treated groups over the control group (Table 6) were observed. No significant differences between the three probiotics treatment groups, in intestinal length or intestinal diameter, were observed.

III. Ileal Mucosa:

The effect of different water probiotics supplementations on the ileal mucosa in 35-day old male broiler chickens are presented in (Table 6). Villus height was significantly greater in all three probiotic treated groups (P ≤ 0.05) than in the control birds. No significant differences were found in crypt depth among the four groups. The villus height/crypt depth ratios in all probiotic treated birds were significantly greater than that of the control ones (P ≤ 0.05). The highest values of villus height and villus height/crypt depth ratio were those of the group received probiotic containing *Lactobacillus acidophilus* plus *Pichia anomola*.

IV. Humoral Anti-ND vaccine antibody titers:

The ability of the probiotics to influence the serum antibody response to vaccination responses against NDV antigen were determined and presented in Table 7. The HI titers against NDV were significantly higher in the 3 studied probiotics groups as compared to the untreated control group, at the 4th and the 5th weeks of age. Moreover, the results indicated that the probiotic + Bacteriophage treated group had significantly higher HI titers than the other probiotic treated groups. Additionally, this treatment group had significantly higher HI titers at the 2nd and the 3rd weeks of age than that of the control group.

Table 3. Effects of water supplementation with probiotics on feed consumption and feed conversion in broilers

Treatment	Feed Consumption (g/bird/day)					Total Feed Consumption (g/bird)
	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	
Lactolife-Av	16.0± 0.05^{b*}	45.2± 0.36^{b}	97.2± 0.70^{ab}	123.6± 1.06	153.9 ± 0.58^{b}	3051.3± 10.66^{ab}
Lactolife-Av+	15.9± 0.07^{b}	44.9± 0.63^{b}	99.7± 1.03^{a}	119.6± 1.52	156.7± 2.44^{ab}	3057.6± 31.33^{ab}
Lactolife-Av+B	15.7± 0.13^{b}	46.9± 0.09^{a}	95.0± 0.84^{b}	122.3± 0.52	151.3± 2.60^{b}	3018.4± 17.44^{b}
Control	16.5± 0.08^{a}	47.2 ± 0.41^{a}	99.2± 1.08^{a}	121.4± 1.99	160.2± 1.88^{a}	3111.5± 25.13^{a}
Probability	0.0001	0.0008	0.0052	0.2471	0.0269	0.0470

Treatment	Feed Conversion (g feed/g body weight gain)				
	0-1 Week	0-2 Weeks	0-3 Weeks	0-4 Weeks	0-5 Weeks
Lactolife-Av	1.191± 0.02	1.093±0.01	1.373± 0.01^{c}	1.495±0.02^{c}	1.638± 0.0^{b}
Lactolife-Av+	1.271± 0.03	1.107 0.01	1.433± 0.02^{b}	1.555±0.02^{ab}	1.654± 0.03^{b}
Lactolife-Av+B	1.228± 0.02	1.115±0.01	1.389± 0.01^{c}	1.535±0.01^{bc}	1.584± 0.04^{b}
Control	1.247± 0.01	1.135±0.02	1.488± 0.02^{a}	1.594± 0.01^{a}	1.737± 0.01^{a}
Probability	0.0523	0.2735	0.0001	0.0060	0.0036

* Means with different superscripts, within trait and age, are significantly different (P ≤ 0.05). -Lactolife-Av = *Lactobacillus acidophilus,* Lactolife-Av+ = *Lactobacillus acidophilus+ Pichia anomola,* and Lactolife-Av+B = *Lactobacillus acidophilus + Pichia anomala* +Bacteriophage

Table 4. Effects of water supplementation with probiotics on mortality rate and production number in broilers

Trait	Mortality Rate (%)						Production number
Treatment	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	Total	
Lactolife-Av	1.33± 0.55	0.67± 0.27^{b*}	1.33 ± 0.55	1.33± 0.55	1.33 ± 0.55	6.00± 0.56^{b}	299.0± 3.90^{a}
Lactolife-Av+	0.67± 0.27	0.67± 0.27^{b}	0.67± 0.27	0.67± 0.27	1.33 ± 0.55	4.00± 0.38^{b}	305.3± 2.88^{a}
Lactolife-Av+B	1.33± 0.55	0.67 ± 0.27^{b}	0.67± 0.27	1.33 ± 0.55	1.33 ± 0.55	5.33± 0.47^{b}	310.6± 6.43^{a}
Control	1.33± 0.55	2.00 ± 0.41^{a}	1.33± 0.55	1.33± 0.55	2.67± 0.90	8.67± 0.58^{a}	263.4± 5.60^{b}
Probability	0.3181	0.0195	0.0731	0.4005	0.7606	0.0056	0.0001

* Means with different superscripts, within column, are significantly different (P ≤ 0.05).
-Lactolife-Av = *Lactobacillus acidophilus*, Lactolife-Av+ = *Lactobacillus acidophilus+ Pichia anomola,* and Lactolife-Av+B = *Lactobacillus acidophilus + Pichia anomala* +Bacteriophage.

Table 5. Effects of water supplementation with probiotics on carcass characteristics in broilers

Treatment	Carcass Characteristics						
	Dressing Weight (%)	Front Part Weight (%)	Hind Part Weight (%)	Breast Meat Weight (%)	Thigh Meat Weight (%)	Carcass Meat Weight (%)	Giblets Weight (%)
Lactolife-AV	69.92± 0.72	39.17± 0.59	30.58± 0.31	16.43 ± 0.29	13.85± 0.24	30.33± 0.31	5.97± 0.15
Lactolife-AV+	69.58± 0.59	39.42± 0.52	30.25± 0.36	16.76± 0.21	14.04± 0.23	30.83± 0.32	5.94± 0.13
Lactolife-AV+B	69.75± 0.34	39.42± 0.48	30.25± 0.23	16.77± 0.13	13.43± 0.18	30.25± 0.26	5.93± 0.14
Control	68.33± 0.37	38.33± 0.34	29.92± 0.25	16.26± 0.25	13.30± 0.21	29.58± 0.39	5.86± 0.11
Probability	0.1460	0.3623	0.4613	0.3105	0.0622	0.0593	0.9425

-No significant differences, within trait, among the treatment groups were observed.
-Lactolife-Av = *Lactobacillus acidophilus*, Lactolife-Av+ = *Lactobacillus acidophilus+ Pichia anomola,* and Lactolife-Av+B = *Lactobacillus acidophilus + Pichia anomala* +Bacteriophage.

Table 6. Effects of water supplementation with probiotics on intestine length and diameter and ileal mucosa in broilers

Trait	Intestinal Morphology		Ileal Mucosa		
Treatment	**Intestine Length (cm)**	**Intestine Diameter (cm)**	**Villus Height (µm)**	**Crypt Depth (µm)**	**Villus Height/Crypt Depth Ratio**
Lactolife- AV	195.25±2.08^{a*}	0.967 ± 0.016^{a}	402± 25^{b}	114± 16	3.53± 0.21^{b}
Lactolife- AV+	196.17± 1.27^{a}	0.967 ± 0.013^{a}	502± 26^{a}	115± 12	4.36 ± 0.12^{a}
Lactolife- AV+B	196.33± 2.64^{a}	0.975 ± 0.012^{a}	400± 19^{b}	110± 15	3.64± 0. 0.18^{b}
Control	184.00 ±2.85^{b}	0.858 ± 0.022^{b}	322± 28^{c}	122± 11	2.64± 0.20^{c}
Probability	0.0003	0.0001	0.0001	0.2102	0.0017

* Means with different superscripts, within trait, are significantly different ($P \leq 0.05$).
-Lactolife-Av = *Lactobacillus acidophilus*, Lactolife-Av+ = *Lactobacillus acidophilus+ Pichia anomola,* and Lactolife-Av+B = *Lactobacillus acidophilus + Pichia anomala* +Bacteriophage.

Table 7. Effects of water supplementation with probiotics on HI titer against Newcastle Disease vaccine in broilers

Treatment	Age				
	1st week	**2nd week**	**3rd week**	**4th week**	**5th week**
Lactolife-Av	2.78±0.27	5.38±0.43^{ab*}	7.22±0.25^{a}	6.50 ±0.60^{b}	5.78± 0.41^{b}
Lactolife-Av+	3.50±0.18	5.30±0.31^{ab}	6.60±0.41^{ab}	6.78±0.34^{b}	5.60± 0.15^{b}
Lactolife-Av+B	3.30±0.23	6.00±0.15^{a}	7.11±0.14^{a}	8.22±0.39^{a}	6.90± 0.26^{a}
Control	3.00±0.25	4.50 ±0.40^{b}	6.00±0.26^{b}	4.80±0.34^{c}	4.40± 0.21^{c}
Probability	0.1556	0.0164	0.0177	0.0001	0.0001

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).
-Lactolife-Av = *Lactobacillus acidophilus*, Lactolife-Av+ = *Lactobacillus acidophilus+ Pichia anomola,* and Lactolife-Av+B = *Lactobacillus acidophilus + Pichia anomala +Bacteriophage*

DISCUSSION

The current study indicates that the administration of probiotics, via the drinking water, had beneficial significant effects on broiler performance, intestinal histomorphological parameters and immune response. The probiotics used in this experiment were monospecies probiotics (*Lactobacillus acidophilus*), multispecies probiotics (*Lactobacillus acidophilus + Pichia* *anomola*) and multispecies probiotics plus a bacteriophage *(Lactobacillus acidophilus* + *Pichia anomala* + Bacteriophage), under the trade names of Lactolife-Av, Lactolife-Av+ and Lactolife-Av+B, respectively. The Lactolife-Av+B had the most beneficial effects on feed consumption; feed conversion ratio and antibody titers against NDV, as compared to the other two probiotics. The final body weight, at 5 weeks of age, increased by all the supplemental probiotics over the control group. In general, probiotic treatments significantly decreased feed consumption improved final feed conversion ratio, and decreased total mortality rate. No significant effects due to supplementation with probiotics were observed on carcass characteristics. These results are in agreement with those reported by many investigators (Karimi *et al*., 2010; Koc *et al*., 2010 and Seyyedmousa, 2011). The major outcomes from using probiotics, in broilers, include improvement in growth (Karimi *et al*., 2010; Koc *et al*., 2010 and Salarmoini and Fooladi, 2011), reduction in mortality (Timmerman *et al*., 2006 and Seyyedmousa, 2011), and improvement in feed conversion efficiency and reduced feed intake (Karimi *et al*., 2010 and Koc *et al*., 2010). However, Mutus *et al*. (2006) and Seyyedmousa (2011) reported that carcass yield in broilers was not significantly affected by probiotics administration.

The positive effects of probiotics on the broiler performance could be attributed to their mode of action. Probiotic is a generic term, and products can contain yeast cells, bacterial cultures or both that stimulates microorganisms capable of modifying the gastrointestinal environment to favor health status, and improve feed efficiency and growth (Fuller, 2001). Mechanisms by which probiotics improve feed conversion efficiency include alteration in intestinal flora, enhancement of growth of nonpathogenic bacteria, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients (Yeo and Kim, 1997).

It is well established that probiotics alter gastrointestinal pH and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients (Lutful, 2009). Supplementation of *Lactobacillus acidophilus* to chickens significantly increased the levels of amylase and produce higher carbohydrase enzyme activities (Jin *et al*., 2000). Dietary supplementation with probiotics resulted in significantly improved protein digestibility (Houshmand *et al*., 2011). Also, Feed supplemented with *Pichia anomola* showed an improved quality due to the addition of advantageous proteins and phytase activity (Kaur and Satyanarayana, 2005 and Volkmar *et al*., 2011). Seyyedmousa (2011) reported that supplementation of yeasts, improved broiler growth, feed conversion ratio and mortality rate through increasing digestibility, decreasing pathogenic microorganism, like *E. coli*, and improving the immune system. It may also be related to a balanced microbial population in the gastrointestinal tract which has an important role in the health and performance of broilers (Koc *et al*., 2010). *Pichia anomala* have been reported to inhibit aflatoxin production by *Aspergillus flavus* (Hua, 2004 and Yin *et al*., 2008) and ochratoxin A production by *Aspergillus ochraceus* (Petersson *et al*. 1998 and Masoud and Kaltoft, 2006). Moreover, feed supplementation with *Pichia anomala* potently reduced ammonia production from poultry manure (Eu *et al*., 2004). Santin *et al*. (2001) also reported significant improvement in body weight and feed conversion for broilers receiving a diet with cell walls of yeast. They suggested that the observed increase in villus height of ileum mucosa, in that study, was a possible explanation for these results. This suggestion was confirmed by the present results, which indicated an increase in villus height in the ileum of the probiotic treated birds.

The positive effects of bacteriophages on the broiler performance may be due to their effect on making the intestine healthier. Bacteriophages are viruses that infect and kill bacteria (Huff *et al*., 2005). Huff *et al*. (2010) reported that bacteriophage therapy significantly reduced the mortality rate in birds challenged with *E. coli*.

The present data indicated significant increase in intestinal length and intestinal diameter in all probiotics treated groups over the control group. The histomorphometric analysis of the ileum revealed increased villus height in all treated groups compared to the control one. These results are consistent with previous experiment of Kabir *et al*. (2005). They demonstrated that broilers fed probiotics had a tendency to display pronounced intestinal histological changes such as active impetus in cell mitosis and increased nuclear size of cells, than the controls. Our results of histological changes support the findings of Samanya and Yamauchi (2002) and Zhang *et al*. (2005). They indicated that birds that were treated with probiotics had a tendency to display greater growth performance and pronounced intestinal histology, such as prominent villus height, extended cell area and consistent cell mitosis, than the controls. Similar results were also reported by Awad *et al*. (2009) who reported that supplementation with probiotics, significantly increased intestinal villus height, which resulted in better performance.

The HI titers against ND vaccine were significantly higher in the 3 studied probiotics groups as compared to the untreated control group. Lactolife–Av+B treatment group had superior figures. Similar significant positive effects of the probiotics on immune response were observed by Kabir *et al*. (2004), Dalloul *et al*. (2005), and Jennifer *et al*. (2011). Haghighi *et al*. (2005) demonstrated that the administration of probiotics enhances serum antibodies to several foreign antigens in chickens. Moreover, Kabir *et al*. (2004) evaluated the dynamics of probiotics on immune response of broilers and they reported significantly higher antibody production in experimental birds as compared to the control ones. Ahmad (2006) suggested that probiotic enhances the immune competence of broilers by macrophage activation, increase of systemic and local antibody production. However, the immunomodulatory activities of probiotic may be related to their ability to induce cytokine production, which leads to regulation of innate and adaptive immune responses (Jennifer *et al*., 2011).

It could be summarized that the mode of action of probiotics in poultry includes: maintaining normal intestinal microflora by competitive exclusion and antagonism (Kabir *et al*., 2005; Kizerwetter-Swida and Binek, 2009), altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (Yoon *et al*., 2004), decreasing feed intake and improving digestion (Awad *et al*., 2006) and stimulating the immune system (Haghighi *et al*., 2005; Jennifer *et al*., 2011).

CONCLUSION

It was hypothesized that the broilers in the Lactolife-Av, Lactolife-Av+ and Lactolife-Av+B probiotic treatments would all have higher body weight, lower mortality, better feed conversion ratio and improved immune response than the broilers in the control group. The results from the current study supported this hypothesis. So, it could be concluded that herein studied probiotics are capable of improving broiler performance and stimulating their immune system, and could be used as natural and safe growth promoters.

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تأثير إستخدام البروبيوتك الأحادى والمتعدد الأنواع على الأداء الانتاجي ومقاييس الهستومورفولجى للامعاء والإستجابة المناعية في بدارى انتاج اللحم

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أجريت هذه التجربة لإختبار مدى جدوى ونفع إستخدام بعض الخلطات المختلفة من البروبيوتك مثل البكتريا النافعة *Lactobacillus acidophilus* (تحت المسمى التجارى Lactolife-Av) – خليط من هذه البكتريا مع خميرة *Pichia anomala* (تحت المسمى التجارى Lactolife-Av+) – أما الخليط الثالث فكان يحوى كلا النوعين السابقين فضلاً عن الفيروسات الممرضة للبكتيريا Bacteriophage (تحت المسمى التجارى Lactolife-Av+B) . وقد أستخدمت هذه الخلطات فى مياه الشرب لبدارى انتاج اللحم لمعرفة مدى تأثيرها على الآداء الإنتاجى – التغيرات المورفولوجية والهستولوجية للأمعاء الدقيقة – فضلاً عن الإستجابة المناعية . تم تقسيم عدد 600 كتكوت تسمين (ذكر) عمر يوم الى اربع مجاميع (كل مجموعة تحتوي علي 6 مكررات). المجموعات من 1 الي 3 تم معاملتهم بمستحضرات البروبيوتك سالفة الذكر علي التوالي عند اليوم الأول و العاشر من عمر الكتاكيت, اما المجموعة الرابعة فكانت مجموعة المقارنة. ولقد أوضحت نتائج التجربة أن مستحضرات البروبيوتك الثلاثة رفعت معنويا من الكفاءة الإنتاجية لبدارى انتاج اللحم طول فترة التجربة (35 يوم) مقارنة بمجموعة المقارنة ، فيما عدا صفات الذبيحة فلم تتأثير معنويا.

ولقد بدا هذا التحسن المعنوي واضحاً بنهاية التجربة حيث لوحظ زيادة فى وزن الجسم (بما يتراوح من 64 – 85 جم/ طائر), إنخفاض معدل إستهلاك العليقة (بما يتراوح من 55 – 105 جم/ طائر) ، تحسن كفاءة التحويل الغذائى (بما يتراوح من 5 – 15 نقطة) كما إنخفض أيضاً معدل النفوق (2,67 – 4,67 %). كما أنه فى نهاية التجربة كانت هذه المجاميع الثلاثة ذات دليل إنتاجى متشابه ، ومتفوقة معنويا عن مجموعة المقارنة. ولقد أوضحت النتائج أيضاً أن تلك الإضافات زادت وبصورة معنوية من طول وقطر الأمعاء الدقيقة فضلاً عن زيادة طول الخملات والنسبة بين طول الخملات وعمق إنخفاضات ليبركون فى منطقة اللفائفى. لكن لم تلاحظ أى إختلافات معنوية بين كافة المجاميع فى عمق إنخفاضات ليبركون. وفيما يتعلق بالإستجابة المناعية المصلية ضد فيروس النيوكاسل فلوحظ زيادة معنوية فى مستوى الأجسام المناعية بمجاميع المعاملة ضد هذا الفيروس عن مجموعة المقارنة فى الأسبوع الرابع والخامس من عمر الطيور.

ختاما يمكن أن نستنتج أن تلك المستحضرات من البروبيوتك يمكن إستخدامها فى بدارى التسمين لرفع الكفاءة الإنتاجية وتحفيز الإستجابة المناعية وأنها مواد طبيعية آمنة الإستخدام كمحفزات للنمو.