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COMPARATIVE STUDY ON THE EFFECT OF PROBIOTICS, ENZYMES, ACIDIFIER OR ANTIBIOTIC GROWTH PROMOTERS ON PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKS IN COMMERCIAL SCALE PRODUCTION .

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ABSTRACT

An experiment was conducted to study the effect of using probiotics, enzymes , acidifiers , or antibiotics in broiler diets against negative control (with no supplemental growth promoters) on growth performance and immune response of Arbor – Acres broiler chicks from 0 – 6 weeks of age. Fifteen thousand (15000) day – old broiler chicks were randomly divided into five experimental treatment groups with two replicates each . The different experimental diets were iso-nutritive and contained 21% crude protein and 2950 Kcal ME/Kg feed during the first four weeks of age (starter / grower period) and contained 17.5% crude protein and 3000 Kcal ME/Kg feed during the last two weeks of age (finisher period) .

At the end of starter / grower period, weight gain and feed conversion values of chicks fed diets supplemented with different growth promoters were approximately similar to those recorded by chicks fed control diet. At the end of experiment (6 weeks), the values of weight gain, feed conversion, performance index and carcass characteristics of birds fed diets containing either probiotics, enzymes, acidifiers or antibiotics had also no significant differences as compared with those fed the control diet. The use of these growth promoters reduced the reisolation percentage of E. coli and proteus organisms from liver and intestine if compared with control group.

The geometric mean titre against Newcastle disease virus was highly in birds which received probiotics followed by those having acidifier and enzymes if compared with other groups.

INTRODUCTION

In the last few years, studies with germ- free chicks and antimicrobial compounds have indicated the significance of the interaction between host nutrition and the intestinal microflora. This interaction led to the decision of the European Union in June, 1999 to remove several antibiotic growth promoters from monogastric diets. These products have been used for many years in poultry diets and have had an effective way of enhancing animal health status, uniformity and production efficiency. The removal of these products (antibiotic growth promoters), and the use of poorly digestible ingredients runs the risk not only of poor performance, which has always been the case, but now there is an additional danger from bacterial overgrowth and subsequent disease / intestinal disorders (Bedford , 2000). Probiotics are non-nutritional additives containing beneficial microbial

cultures and/or ingredients that enhances growth of desirable gastrointestinal microbes of the host animal (Marionnet and Lebas ,1990) . Supplementation of these probiotics stimulates the activity of certain important bacteria which are involved in the digestive processes , protein synthesis and nutrient absorption in the gastrointestinal tract (Stockland 1993). The mechanism of probiotics effect on animal health was conclusively reviewed by Jin *et al* ., (1997) who concluded that probiotics enable the host animal to return to normal through increasing normal gut flora on the expense of pathogenic organisms . Many studies have been conducted to test the efficacy of such preparationts on animal growth and performance. Several studies with broiler have indicated that probiotic preparations improve live weight gain and feed conversion rate, and markedly reduce mortality (Jin *et al*., 2000). However , a number of studies have shown that probiotics have no positive effects on broilers (Buenrostro and Kratzer , 1983 ., Watkins and Kratzer 1984 and Maiolino *et al*.,1992).

The most important action of enzymes in gastrointestinal tract of poultry is the disruption of cell walls in the feed particles and increase apparent nutrient availability. This in turn allows better absorption of energy from fats as well as carbohydrates, and improves nitrogen utilization (Pack, 1996 and Clifford,1998).

The acidifiers or organic acids such as acetic, propionic , formicetc are produced by the normal anaerobic intestinal flora as side products of their metabolism (Mead , 2000). Volatile fatty acids (VFA'^{s}) or short chain fatty acids ($SCFA'^{s}$), mainly acetic, propionic and butyric, can be added directly to the feed .These acids (acidifiers) not only exert an antibacterial effect in the intestine, but also in the crop (Hinton and Linton , 1988). Many of the recently developed products including organic acids as well as probiotics do preferentially target bacteria of the gram - negative flora , which is in fact desirable (Engberg and Petersen , 2001)

The Objective of this experiment is to study the effect of using probiotics , enzymes , acidifiers or antibiotic growth promoter in broiler diets on growth performance and immune response, in commercial scale production.

MATERIALS AND METHODS

An experiment was carried out at EL - Motaheda Poultry Company during February and March 2000 to evaluate, in commercial scale production, the performance of broiler chicks fed diets supplemented with probiotics, enzymes, acidifiers or antibiotic. A total number of 15000 one - day -old unsexed Arbor Acres broiler chicks of nearly similar live body weight (40 gram) were randomly distributed into 5 treatment groups; each contained 3000 birds in two replicates. Chicks were allocated in a littered floor poultry houses in an open system under the same management conditions. Water and feed were offered ad-libitum and artificial lighting was provided 24 hours daily all over the experimental period, which lasted for 6 weeks . All groups were received a routin vaccination against Newcastle disease (ND), infectious bursal disease (IBD) and infectious bronchitis (IB).

The composition and calculated chemical analysis of the experimental diets are shown in Table (1). The control group was fed on diets (1) which had no supplemental growth promoter and contained 21 % crude protein and 2950 Kcal ME/ Kg feed during first four weeks of age (Starter/Grower period) and contained 17.5% CP and 3000 Kcal ME/ Kg feed during the last two weeks of age (Finisher period). The other groups were fed on diets similar to those used in control group (1), except that groups diets (2) and (3) were supplemented either with probiotics or enzymes mixture, respectively at inclusion rate of 1 Kg / ton of feed (0.1 %). However, diets of group (4) were supplemented with 2 Kg acidifiers/ton feed (0.2%). As for group (5) it were supplemented with 100 grams Flavomycin per ton of feed as antibiotic growth promoter(o.o1%). The compositions of these supplements are:

1 – Probiotic (group 2): Bacillus Licheniformis, Bacillus subtilis.

2 - Enzymes mixture (group3): amylase, protease and xylanase.

3 - Acidifiers (group 4): Fumaric, citric, malic, sorbic, and tartaric acids.

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

Ingredients %	Treatments									
	Starter/Grower					Finisher				
	1	2	3	4	5	1	2	3	4	5
Yellow Corn	60.00	60.00	60.00	60.00	60.00	71.0	71.0	71.0	71.0	71.0
Soybean meal (44%)	30.00	30.00	30.00	30.00	30.00	21.0	21.0	21.0	21.0	21.0
Corn gluten meal(62%)	5.000	5.000	5.000	5.000	5.000	4.0	4.0	4.0	4.0	4.0
Di- calcium phosphate	1.943	1.943	1.943	1.943	1.943	1.915	1.915	1.915	1.915	1.915
Lime stone	1.213	1.157	1.157	1.057	1.213	1.234	1.134	1.134	1.034	1.224
Vegetable oil	1.000	1.000	1.000	1.000	1.000	-	-	-	-	-
Na Cl	0.444	0.400	0.400	0.400	0.434	0.386	0386	0.386	0.386	0.386
DL - methionine	0.050	0.050	0.050	0.050	0.050	0.070	0.070	0.070	0.070	0.070
L- Lysine HCL	0.050	0.050	0.050	0.050	0.050	0.095	0.095	0.095	0.095	0.095
Vit . & Min.Mixture*	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0300	0300	0.300
Probiotic	-	0.1	-	-	-	-	0.1	-	-	-
Enzymes mixture	-	-	0.1	-	-	-	-	0.1	-	-
Acidefiers	-	-	-	0.2	-	-	-	-	0.2	-
Antibiotic (Flavomycin)	-	-	-	-	0.01	-	-	-	-	0.01
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis :**										
Crude protein %	21.48	21.48	21.48	21.48	21.48	17 .89	17.89	17.89	17.89	17.89
ME (Kcal / Kg diet)	2958	2958	2958	2958	2958	3002	3002	3002	3002	3002
Calcium %	0.99	0.97	0.97	0.93	0.99	0. 97	0.97	0.97	0.97	0.97
Available phosphorus%	0.50	0.50	0.50	0.50	0.50	48	0.48	0.48	0.48	0.48
Methionine %	0.42	0.42	0.42	0.42	0.42	0. 37	0.37	0.37	0.37	0.37
Methionine + Cystine %	0.78	0.78	0.78	0.78	0.78	0. 70	0.70	0.70	0.70	0.70
Lysine %	1.06	1.06	1.06	1.06	1.06	0. 87	0.87	0.87	0.87	0.87
N a %	0.18	0.17	0.17	0.17	0.17	0. 17	0.17	0.17	0.17	0.17
EE %	3.65	3.65	3.65	3.65	3.65	2.97	2.97	2.97	3.97	2.97
CF %	3.49	3.49	3.49	3.49	3.49	3 .08	3.08	3.08	3.08	3.08

***Contains: Vit.A 12 mIU ; Vit D_3 2.2 mIU ; Vit.E 10g ; Vit.K 2g ; Vit B_1 1g ; Vit.B_2 5g ; Vit B_6 1.5 g ; Vit B_{12} 10mg ; Niacin 30g ; pantothenic acid 10g ; Folic acid 1g ; Biotin 50mg ; Choline 300g ; Iron 30g ; Iodine 1g ; Zinc 50g ; Manganese 60g ; Copper 4g ; Selenium 100 mg ; Cobalt 100 mg .**

****According to NRC (1994)**

Data on live body weight, feed consumption, feed conversion and mortality rate were recorded. Performance index (PI) was calculated according to North (1981) as follows:

PI = (Live body weight (Kg) / feed conversion) x 100.

At the end of the experiment, all birds were fasted for 12 hours, weighed and slaughtered to determine the dressing and giblets weight. At the

same time, 5 chicks from each group were sacrificed for a trial of E.coli and Proteus reisolation from livers and intestines occurred according to Cruickshank (1975). Suspected microbial colonies were tested serologically by antisera.. Blood samples were collected from all groups weekly to determine the antibody (Ab) titer against Newcastle disease according to the method of Reed and Muench(1938).

Data were statistically analyzed using the linear model (SX, 1992). A simple one - way classification analysis followed by least significant difference test (LSD) were used for testing the significance between means.

RESULTS AND DISCUSSION

The performance data obtained in this study at the end of starter / grower (the 28^{th} day of age) and at the end of the experimental period (the 42^{th} day of age) are summarized in Table (2). It appeared that, no significant differences were detected in live body weight, feed intake, feed conversion and performance index among different dietary treatments either during starter / grower, finisher period (28 -42 days of age) or the whole experimental period. These results are in agreement with those reported by Buenrostro and Kratzer (1983), Watkins and Kratzer (1984) and Maiolino *et al.*, (1992) who found no significant differences in final body weight and feed conversion ratio of broiler chicks fed diet supplemented with probiotics. However, Jin *et al.*, (2000) concluded that the addition of probiotics to the diet has been found to improve growth performance and feed conversion in broilers . Variation in the effects of probiotics on chicks obtained from various studies may be attributed to the differences in the strains and forms of bacteria used and in their concentrations of dietary supplements. In most of the studies the source of the microbials in the probiotics is not reported. The lack of consistency in the results has caused many people to be sceptical about the positive effects of probiotic in chicks. Makled (1991) concluded that restoring gut flora as a result of ingesting large quantities of specific bacteria through feeding probiotics enabled the host animal to return to normal. This phenomena of increasing normal gut flora on the expense of pathogenic organism was gain the term "competitive exclusion" (Ziprin and Deloach , 1993).

It is worth to mention depending upon data obtained that the addition of enzymes preparation to commercial diets in feeding broilers had no significant effects on live body weight, weight gain, feed intake and feed conversion at marketing age as was reported by Mervat (1999). Enzymes supplementation is used to improve economic effectiveness, but the result depend both on the enzymes as well as substrate used (Mikulski *et al.*, 1997).

Data also revealed that the acidifiers treatment had no significant effect on body weight gain and feed conversion ratio at marketing age. In most experiments where single VFA was added to the feed, no protection against salmonella was found (Hume *et al* ;1993). The SCFA diffuse into the bacterial cell in undissociated form. Inside the bacterial cell, the acid dissociated, resulting in reduction of intracellular PH and anion accumulation (Van der Wielen *et al* ., 2000).

Table (2): The effect of some feed additives on the performance of broiler chicks and carcass traits.

Item	Treatments					Sign
	Control (1)	Probiotics (2)	Enzymes (3)	Acidifier (4)	Antibiotic (5)	
At 4 Weeks of age:						
Live body weight (g/bird)	865	855	875	825.5	847.5	N.S.
Feed consumption (g/bird)	1648.8	1626.5	1658.3	1612.1	1625.5	N.S.
Feed conversion	1.906	1.902	1.895	1.902	1.918	N.S.
Performance index (PI)	45.38	44.95	46.17	44.82	44.19	N.S.
4-6 Weeks of age:						
Live body weight (g/bird)	1542	1564.5	1559.5	1550.5	1534.5	N.S.
Body weight gain (g/bird)	677	709.5	684.5	698.0	687	N.S.
Feed consumption (g/bird)	1987.2	2049	1958.8	2026.9	2028	N.S.
Feed conversion	2.935	2.889	2.861	2.904	2.952	N.S.
Performance index (PI)	23.07	24.56	23.93	24.04	23.57	N.S.
At 6 Weeks of age:						
Live body weight (g/bird)	1542	1564.5	1559.5	1550.5	1534.5	N.S.
Feed consumption (g/bird)	3636	3675.5	3617	3648	3654	N.S.
Feed conversion	2.358	2.349	2.319	2.353	2.381	N.S.
Performance index (PI)	65.39	66.60	67.25	65.89	64.45	N.S.
Carcass traits at 6 Weeks:						
* Dressing weight (g/bird)	1049.5	1072.0	1066.7	1057.3	1044.1	N.S.
% of live body weight	68.06	68.52	68.40	68.19	68.04	N.S.
** Giblets weight (g/bird)	66.4	67.6	67.4	67.1	66.3	N.S.
% of live body weight	4.31	4.32	4.32	4.33	4.32	N.S.
Total edible ports (g//bird)	1115.9	1139.6	1134.1	1124.4	1110.4	N.S.
% of live body weight	72.37	72.84	72.72	72.52	72.36	N.S.

*** Dressing = The front parts with wings, hind parts and the neck.**
**** Giblets = The heart, empty gizzard and liver.**
N.S. = Not significant.

The use of antibiotic growth promoters in broiler chicks diets were reported by various workers (Ghazalah *et al.*, 1994 and Noh *et al* ., 1994) . Supplementing broiler diets by falvomycin have no significant improving effect upon body gains and feed conversion (Plaur *et al* ., 1983 and Hataba *et al.*, 1997). However, Ghazalah *et al*, (1994) found that the addition of flavomycin to chick diets resulted in an increase in live weight. There are contradictory results of antibiotics response which partly could be due to type of diet (type or source of protein), environmental conditions which mainly related to temperature or heat stress and physiological status of bird concerned (Aly *et al* ., 1985). Moreover, Attia *et al.*, (1997) found that there was no significant effect of pronutrients under investigation (antibiotics, probiotics and enzymes) on body weight, feed consumption and feed conversion ratio during the experimental period.It could be concluded that under the conditions of this experiment as the producers, in commercial scale poultry production, use antibiotics in diseases treatments , the effect of

antibiotics growth promoter, probiotics, enzymes or acidifiers uses was not clear. That may be due to the mortal effect of high dose of antibiotic on all types of bacteria either harmful or useful.

The effect of different treatments on carcass characteristics (as percentages of live body weight) are shown in Table(2). Results indicated that the average values of dressing and giblets percentages were nearly similar and there was no clear trend due to the different treatments. These results are in agreement with those obtained by Ghazalah *et al.*, (1994) who reported that the dressing percentage and total edible parts were not significantly affected by the addition of flavomycin.Similarly, Abd el - Azeem (2002) showed that, dressing and total edible parts percentages insignificantly increased in experimental groups fed diets supplemented with biological feed additives. Data also revealed that the enzymes treatment had no influence on carcass weight and dressing percentage. These results agree with those of Ghazalah *et al* (1994). Olso, Mervat (1999) found that different supplementations (enzymes, antibiotics or probiotics) had no beneficial effect on carcass characteristics .

Regarding to the effect of using probiotics, Enzymes, acidifiers , and antibiotics in comparatively, on reisolation of E.coli and proteus organisms, it was found that probiotics, enzymes and acidifiers followed by antibiotics reduced the reisolation percentage of these organisms from liver and intestine if compared with control groups (Table 3). These result accord with the finding of kutkat *et al*;(2002) who reported that probiotic (Lactobacillus acidophilus) completely eliminated E.coli and clostridium perfringens when used prophylactically for 10 days before infection. The investigators found that the percent of inhibition was decreased to 70 % and 40 % for E.coli and clostridium perfringens, respectively when lactobacillus was added to ration for 3 days before infection. Shoeib *et al.*, (1996) suggested that regulation of the microbial environment of the intestine, may lead to inhibition of the pathogenic intestinal micro organism.

Table (3): Effect of some feed additives on reisolation of E.coli and proteus .

Treatments	Results of reisolation											
	Liver						Intestine					
	E.coli			Proteus			E.coli			Proteus		
	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%
(1) Control	5	4	80	5	3	60	5	5	100	5	5	100
(2) Probiotic	5	0	0	5	1	20	5	0	0	5	0	0
(3) Enzymes	5	0	0	5	0	0	5	1	20	5	0	0
(4) Acidifier	5	1	20	5	0	0	5	1	20	5	0	0
(5) Antibiotic	5	4	80	5	0	0	5	4	80	5	0	0

Results in Table (4) showed that the geometric mean titer (GMT) against Newcastle disease virus one week post vaccination was higher in birds which treated by enzymes followed by acidifiers and probiotics. Slight difference was observed between birds which treated by antibiotics and control group. After 2,3 and 4 weeks post vaccination, the GMT was highly in birds which received probiotics followed by acidifiers and enzymes if compared with other Groups (Table 4).

Table (4): Result of antibody response against Newcastle disease vaccine virus (NDVV).

Treatment	Sampling after vaccination			
	1 Week	2 Week	3 Week	4 Week
(1) Control	2.1^{b}	3.6^{bc}	4.0^{bc}	4.0^{bc}
(2) Probiotics	2.4^{ab}	4.7^{a}	5.3^{a}	5.4^{a}
(3)Enzymes	2.9^{a}	3.8^{abc}	5.0^{ab}	5.1^{ab}
(4)Acidifier	2.5^{ab}	4.6^{ab}	5.4^{a}	5.3^{a}
(5)Antibiotic	2.0^{b}	3.4^{c}	3.2^{c}	3.2^{c}

a,b,c: Means with different letters in the same column are significantly different ($p< o.o5$).

Lactobacillus spp. have been shown to produce digestive enzymes in vitro and the enzymes may enrich the concentration of intestinal digestive enzymes . Szylit *et al.,* (1980) reported that two out of five strains of lactobacillus isolated from male chicks had α amylase activity. The results in Table (4) were in agreement with, Kemin production Manual (1990). On the other hand Dunham *et al.,* (1993) reported that birds treated with lactobacillus reuteri exhibited longer ileal villi and deeper crypts which is a response associated with enhanced T cell function and increase production of IgM against salmonella . Nahashon *et al.*, (1994) found that lactobacillus supplementation of layers increased cellularity of peyer's patches in the ileum which increase a stimulation of the mucosal immune system. Immune modulation of acidifiers was studied by Awaad *et al.*, (2000).They found that acidifiers strongly sustained the production of antibodies against sheep RBes red blood cells; on the other hand acidifiers improve the level of serum transformin.

Finally it could be concluded that; the usage of these feed additives (probiotics, enzymes, acidifier and antibiotic) in broilers feeding gave a promising results in controlling of E. coli and proteus micro organisms as well as the post vaccinal reactions against NDV. However the effect of these additives is not clear on growth performance under the condition of these experiment. It may duo to recurrent the use of antibiotics as a prophylactic in the broiler flocks. These results recommended that a further investigation on the use of these additives.

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مقارنة تأثير استخدام كل من منشطات النمو الطبيعيـــة، الإنزيمـــات، الأحمـــاض العضوية أو المضادات الحيوية على الأداء الإنتاجي والاستجابة المناعيـــة لدجـــاج اللحم

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أجريت تجربة لدراسة تأثير استخدام كل من منشـــطات النمـــو الطبيعيـــة (بروبيوتـــك) والانزيمات والأحماض العضوية بدلاً من المضادات الحيوية في علائق دجاج اللحم على المظـــاهر الإنتاجية وصفات الذبيحة والاستجابة المناعية. استخدم ١٥ ألف كتكوت "أربور ايكـــرز" قســـمت عشوائيا إلى خمسة مجموعات تمثل كل مجموعة معاملة غذائية وبكل منـــها ٣٠٠٠ طـــائر (فـــي مكررين بكل منها ١٥٠٠ طائر). استخدمت المجموعة الأولى (المقارنة) علائق تجريبية تحتـــوي على ٢١% بروتين، ٢٩٥٠ كيلو كالوري/كجم في فترة البادي/نامي (من عمر يوم إلى عمـــر ٢٨ يوم) وعليقة تحتوي على ١٧,٥% بروتين، ٣٠٠٠ كيلو كالوري/كجم في فترة الناهي (من٢٩ إلى ٤٢ يوما)، غذيت باقي المجموعات على علائق مشابهة لعلائق مجموعـــة المقارنـــة مـــن حيـــث محتواها من المركبات الغذائية ولكن استخدم فيها ٠,١% بروبيوتك أو إنزيمـــات فـــي المجموعـــة الثانية أو المجموعة الثالثة على التوالي بينما استخدم ٠,٢% أحماض عضويـــة فـــي المجموعـــة الرابعة. أما المجموعة الخامسة فتم استخدام مضاد حيوي (فلافومايسين) بنسبة ٠,٠١%.

أوضحت النتائج سواء عند عمر ٢٨ يوما أو في نهاية التجربة (عمر ٤٢ يوما) أنـــه لا يوجد فروق معنوية بين المجموعات المختلفة في كل من وزن الجسم ــ الاســـتهلاك الغذائـــي ــ معامل التحويل الغذائي وكذا دليل كفاءة النمو (PI) وصفات الذبيحة. في حين لوحظ أن اســـتخدام الإضافات الغذائية المختلفة أدى إلى تقليل النسبة المعزولة من بكتريا E-Coli، Proteus من الكبد والأمعاء وذلك عن مجموعة المقارنة. كذلك كانت الأجسام المناعية ضد مرض التيوكاسل مرتفعـــة في المجموعات التي غذيت على منشطات النمو الطبيعية يليها المغذاة على الأحمـــاض العضويـــة والإنزيمات مقارنة بباقي المجموعات.