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Comparison of the Effects of Three Different Types of Probiotics on the Alkaline Phosphatase Activities of the Small Intestine Mucosa of Broiler Chicks

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Abstract: An experiment was conducted to study the effects of different types of probiotic on alkaline phosphatase activities of the small intestine mucosa of broilers. The experimental design was arranged as randomized completely blocks in 4 × 2 factorial arrangement of treatment. 180 male broilers of Ross 308 commercial hybrid were designated into 4 groups. Three replicates of 15 birds were assigned to each treatment. Control treatments (diet contained no probiotic) were fed according to the NRC as base diet and three treatment groups were fed from the same diet plus three different types of probiotics. Birds were slaughtered after 21 and 42 days and different segments of small intestine(at 1, 10, 30, 50, 70 and 90% of total length the small intestine) were taken from e ach replicates (N=2) Alkaline phosphatase enzyme activities were measured and recorded. Obtained data were analyzed by Spss ($P<0.05$). In three treatment groups probiotic, significantly increased alkaline phosphatase (ALP) activities at 21days of age at different sites along the small intestine of the broiler chicks ($P<0.05$), these data suggested that probiotics administration have significant effect on treatments comparing to control group.

Key words: Probiotics · Alkaline Phosphatase · Small Intestine · Broiler Chicks

INTRODUCTION

Probiotics are some additives and directly fed-microbial populations can be added directly to food to balance intestinal microflora and microbial population can to extent prevent intestinal some infections, can have positive effect on animal performance and improve and increase growth of livestock [1]. Probiotics are different from antibiotics and these micro-organisms are live and not contain certain chemical molecules. Probiotics have not residual tissue and create no microbial resistance [2].

Characteristics of an Ideal Probiotic:

- It should be capable of exerting a beneficial effect on the host animal, e.g. increasing growth or increasing resistance to disease.
- It should be non-pathogenic & non toxic.
- It can inhibit gram positive & gram negative germs (CE).
- It should be present as viable cells, preferably in large numbers.
- It should be capable to survive and metabolize in the gut environment, e.g. resistant to low pH and organic acids.
- It should be stable and capable to remain viable for long periods under storage and field conditions.
- It can not compete for nutrient utilization [3, 4].

Aim of this subject was to clarify the change level in the activity of enzyme alkaline phosphatase due to consumption of probiotic food in the intestines.

MATERIALS AND METHODS

Birds and Diets: One hundred eighty male broilers of commercial hybrid (Ross 308) were designated into 4 groups. Three replicates of 15 birds were assigned to each treatment. The birds were kept separately in cages next to each other and on the litter. All conditions for groups were same except mentioned control group was fed according to NRC recommendations [5] from a basal diet with no probiotic and the treatment groups were fed by basal diet containing three species of probiotics

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Table 1: Ingredients and nutrients composition of experimental diets

	(1-21 Days)			(21-42 Days)		
	0%	2%	4%	0%	2%	4%
Ingredient						
Corn	54.50	54.00	45.00	62.64	39.00	39.00
SBM[1] (%44)	34.14	34.19	35.81	27.00	27.70	27.70
Oil	2.50	2.50	2.50	2.50	2.50	2.50
Methionine	0.60	0.60	0.80	0.60	0.60	0.60
Lysine	0.00	0.00	0.00	0.20	0.20	0.20
Vitamin-premix	0.25	0.25	0.25	0.25	0.25	0.25
Mineral-premix	0.25	0.25	0.25	0.25	0.25	0.25
DCP[2]	1.60	1.60	1.62	1.13	1.13	1.13
Oyster	1.44	1.40	1.33	1.48	1.44	1.44
Salt	0.28	0.28	0.28	0.28	0.28	0.28
probiotic	0.00	2.00	4.00	0.00	2.00	2.00
Starch	1.06	1.41	7.37	0.00	2.60	2.60
Fine Sand	3.38	1.46	0.07	3.67	2.05	2.05
Nutrients						
ME[3] (kcal/kg)	2850.21	2850.11	2850.14	2920.54	2920.03	2920.03
Protein (Percent)	20.50	20.51	20.50	18.17	18.18	18.17
Calcium (Percent)	0.99	0.99	0.99	0.89	0.89	0.89
Phosphorus (Percent)	0.44	0.44	0.44	0.34	0.34	0.34
ME/Protein	139.00	138.96	139.03	160.69	160.64	160.64
Calcium/ Phosphorus	2.23	2.23	2.23	2.56	2.58	5.58

SBM1 = soybean meal; DCP[2] = dicalcium phosphate. ME[3] = Metabolisable energy. Per 2.5 kg mineral supplement containing 99200 mg magnesium, 84700 mg zinc, 50000 mg iron, 10000 mg copper, 990 mg Iodine, 200 mg selenium, 250000 ml gram Colin chloride. Per 2.5 kilogram vitamin supplement containing 900000 IU of vitamin A, 200000 IU of vitamin D_3, 19000 IU of vitamin E, 200 mg vitamin K_3, 18050 mg vitamin B_1, 49000 mg vitamin B_2, 9800 mg vitamin B_3, 29650 mg vitamin B ,5 2940 mg vitamin B ,61000 mg vitamin B , 15 mg vitamin B , 100 mg biotin, 190000 mg cholin chloride, 1000 mg antioxidant

($T_{1\ =Protexin}$, $T_{2=Bioplus\ 2B}$, $T_{3=Biosaf}$). Diets were prepared according to NRC [5] and during the first 21 days of life and from 22 to 42 days by a starter and a finisher diet adlibitum [5].

Sample Collection: In the Rearing period, all conditions such as temperature, humidity, light, ventilation and management were appropriated and similar for all broilers and in days 21 and 42 of the rearing period, after 5 hours of starvation, 2 broilers from every group which weighed nearly equal to the average weight of each replicate have been chosen and slaughtered. The abdominal cavity was opened and the entire gastrointestinal tract was removed. The small intestine was isolated and the length of intestine was determined by a graduate ruler. The positions at 1, 10, 30, 50, 70 and 90 % of the length of small intestine for analyzing the ALP enzyme activity were separated with specific scissors (a 8-cm sample was taken). The samples for ALP determination were cut open lengthwise, rinsed carefully with phosphate buffer saline (pH=7), blotted dry, then samples envelop in vacuum packed and stored at -80°C until enzyme analysis [6, 7].

Enzyme Assay: After thawing treatment, all samples were opened and then using a sensitive scale, 0.05 gram of the mucosal small intestine was weighed and along with 10 ml liter phosphate buffer saline (pH=7) was formed into a homogenized solution using sonic Vibracell Sonics (VCX 130 TE USA) device [6, 7]. The activity of ALP was determined according to the procedure of Dahlqvist and Thamson [8] and Ghiasi *et al.* [6, 9] (In this method used from paranitrophenyl with special purity as substrate. With effect of alkaline phosphatase, paranitrophenyle that is colorless decomposed consequently paranitrophenyl were produced that in alkaline pH make yellow color and color severity is related directly with this enzyme activity. For measuring the activity of ALP, It was needed to determine total protein in which (calorimetric) method was used [6, 9]. The activity level of ALP enzyme of each sample is divided into the amount of its total protein. Therefore, the activity level of the enzyme, according to the IU /gram protein is researched [6, 7, 9].

Statistical Analyses: The results of the experiment was analyzed and by Multivariate Analysis of Variance by using the linear model of SPSS software [10].

Table 2: Comparison of average ALP activity between treatment and control groups in different periods and segments of small intestine in broiler chicks (IU/g protein)

		% length of small intestine					
Age	Different Type of Probiotic	1 %	10 %	30 %	50 %	70 %	90 %
21	Control (a)	84242.238^{abcd}	62600.336^{acd}	62362.185^{acd}	54200.616^{abcd}	25110.111^{abcd}	9363.8434^{abcd}
	Protoxin (b)	101050.48^{abd}	109462.42^{bd}	111231.60^{b}	28556.557^{abcd}	17236.653^{abcd}	3247.4323^{abcd}
	Bioplus 2 (c)	52645.668^{acd}	38886.020^{acd}	76157.007^{acd}	34412.173^{abcd}	21972.507^{abcd}	5780.0285^{abcd}
	Biosaf (d)	66728.350^{abcd}	88215.062^{abcd}	79127^{acd}	42074.060^{abcd}	17907.646^{abcd}	7541.5370^{abcd}
42	Control (a)	128421.72^{abcd}	63913.846^{abcd}	98997.508^{abcd}	84181.217^{abcd}	19889.523^{abcd}	14921.073^{abcd}
	Protoxin (b)	80365.605^{abcd}	90120.354^{abcd}	65195.531^{abcd}	25965.136^{abcd}	23843.774^{abcd}	7604.9545^{abcd}
	Bioplus 2(c)	158939.41^{abcd}	135220.05^{abcd}	157960.02^{abcd}	42101.055^{abcd}	7314.0700^{abcd}	6293.7756^{abcd}
	Biosaf (d)	150864.79^{abcd}	61712.293^{abcd}	62173.196^{abcd}	23630.532^{abcd}	16402.655^{abcd}	8104.0706^{abcd}

** a,b,… Means in the same column with different superscripts differ significantly X ± SD (P<0.05)

Analysis of variance according to the model,

$$xij = \mu + Tj + eij$$

Where,

xij = All dependent variable
μ = Overall mean
Ti = The fixed effect of RRO levels (i = 1, 2, 3)
Eij = The effect of experimental error

Values of different parameters were expressed as the mean ± standard deviation (X±SD). There are significant differences between obtained means. Means were analyzed using Duncan's multiple range tests.

RESULT AND DISCUSSION

According to Table 2, adding probiotic to the diet of the broilers at different ages and parts of the small intestine caused variety of influences on the activity of ALP enzyme. In a way that the activity of ALP enzyme demonstrated a significant increasing only at the age of 21 days after sampling as 1%,10%, 30% of the length of the small intestine in treatment groups comparing to control treatment was showed (P<0.05). Probiotices, have significant effect on ALP activity in different ages and segments of small intestine [6].

Microorganisms mostly colonize in birds in the crop, cecum and partly small intestine. They colonized in the small intestine are acquainted with their environment soon; they are mostly of the bacillus type. In order to settle for a long time, they have to stick to the surfaces of the villi, so it seems logical that they can produce some change on the small intestine villi [11]. Villi are more flat and leaf shape in herbivores, whereas in carnivores birds these villi are tall and finger shape [12] villi are covered with enterocytes which are responsible for absorption of food material [12]. Hampson [13] believed that measurements of the villus height and their shape can give us an indication of the enterocyte numbers [13]. According to Ghiasi *et al.* [6] any change in villus height leads to a change in absorption rate [6, 14].

Alkaline phosphatase is a group of enzymes have catalytic activity for degradation of phosphate esters and separation of phosphoric acid molecules [15, 16]. In mammalians and chicken intestinal mucosal cytosole at brush border ends have considerable alkaline phosphatase activity [17]. Moog [15] reported that intestinal epithelium of chicken embryo didn't has alkaline phosphatase earlier day 8 of embryonic life and alkaline phosphatase become activate after day 9-18 [18]. Moog [19] reported, alkaline phosphatase activity can arrive to peak at 2 or 2.5 day post-hatch. Studies show that alkaline phosphatase activity indicates maturity of intestinal cells and had key role in long chain fatty acids and cholesterol digestion [20].

It had been reported that using molds, as a probiotic to mice feed had increased brush–border enzyme activities like sucrase, alkaline phosphatase and leucine aminopeptidase [21, 22].

CONCLUSION

Adding probiotics to the diet, showed a significant elevation in alkaline phosphatase. In the proximal small intestine of broiler chicken by the end of the 3rd week probiotics had affected the villous dimension too so that both villous height and crypt deep had increased and this is in agreement with our findings in the activities of alkaline phosphatase which is probably because an increasing in the cell population therefore It can be concluded that probiotics can enhance the efficiency of digestion and absorption mostly by the end of the first month of production in broilers.

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