International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:6, No:7, 2012

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

Fazlollah Moosavinasab, Zhila Motamedi

***Abstract*—**An experiment was conducted to study the effects of different types of probiotic on Sucrase enzyme activity of the small intestine mucosa in male 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 each replicates (N=2) Sucrase enzyme activities were measured and recorded. Obtained data were analyzed by Spss ($P<0.05$). In three treatment groups, probiotic had no significant effect on sucrase activity in different ages and segments of small intestine ($P<0.05$). These data suggested that probiotics administration had no significant effect on treatments comparing to the control group.

***Keywords*—**Broiler, Chicks, Probiotics, Small Intestine, Sucrase

I. Introduction

PROBIOTICS are additives and directly fed-microbial populations which can be added directly to food to balance intestinal microflora and microbial population. It can prevent intestinal infections to some extent, has positive effect on animal performance and improves and increases the growth of livestock [1]. The main purpose of using probiotics in the diet of chickens is to gain weight and improve feed conversion [2]. Improving intestine function due to the strong interaction between probiotics and gut microflora can be attributed to the following reasons:

1. Reduction in the regular utilization of nutrients by micro-organisms.
2. Reduction of microbial metabolites that interfere with the growth of their hosts [3].

The decrease in metabolites of intestinal pathogens causes the intestinal cells to reduce turnover and thus more energy would be available for production. Finally, the use of probiotics reduces opportunistic pathogens and non-clinical infections [4]. Probiotics are different from antibiotics, and these micro-organisms are live and do not contain certain chemical molecules. Probiotics do not have residual tissue and create no microbial resistance [5].

Fazlollah Moosavinasab is with Departement of Veterinary, Behbahan Branch, Islamic Azad University, Behbahan, Iran (phone: 0098-916-1711172; e-mail: fzmoosavi@gmail.com)
Zhila Motamedi is with Management expert(e-mail: zhila.motamedi@yahoo.com)

A. Characteristics of an Ideal Probiotic

1. It should be capable of exerting a beneficial effect on the host animal, e.g. increasing growth or increasing resistance to disease.
2. It should be non-pathogenic & non toxic.
3. It can inhibit gram positive & gram negative germs (CE).
4. It should be present as viable cells, preferably in large numbers.
5. It should be capable to survive and metabolize in the gut environment, e.g. resistant to low pH and organic acids.
6. It should be stable and capable to remain viable for long periods under storage and field conditions.
7. It cannot compete for nutrient utilization [3], [6].

The aim of this subject was to clarify the change level in the activity of enzyme Sucraze due to consumption of probiotic food in the intestines.

II. Materials And Methods

A. 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 the same except mentioned control group was fed according to NRC recommendations [7] from a basal diet with no probiotic and the treatment groups were fed by basal diet containing three species of probiotics ($T_{1\ =Protexin}$ ،$T_{2=Bioplus\ 2B}$،$T_{3=Biosaf}$). Diets were prepared according to NRC [7] and during the first 21 days of life and from 22 to 42 days by a starter and a finisher diet adlibitum [7].

B. Sample collection

In the Rearing period, all conditions such as temperature, humidity, light, ventilation and management were appropriated and similar for all broilers. 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 sucrase enzyme activity were separated with specific scissors (an 8-cm sample was taken). The samples for sucrase determination were cut open lengthwise, rinsed carefully with phosphate buffer saline (pH=7), blotted dry, the samples were then envelop in vacuum pack and stored at −80°C until enzyme analysis [8], [9]

TABLE I
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 (%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	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 (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

SBM = soybean meal; DCP = 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_6, 1000 mg vitamin B_9, 15 mg vitamin B_{12}, 100 mg biotin, 190000 mg cholin chloride, 1000 mg antioxidant.

C. 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 [8], [9]. The activity of sucrase was determined according to the procedure of Dahlqvist [10], Hill [11] and Teshfam [12]. It goes without mentioning that for measuring the activity level of sucrase. For measuring the activity of sucrase, it was needed to determine total protein in which pirogallol (calorimetric) method was used [12]. The activity level of sucrase 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 [8], [9], [13].

D. Statistical Analyses

The results of the experiment was analyzed and by Multivariate Analysis of Variance by using the linear model of SPSS software [14]. Comparative analysis of the average of treatments was performed using Duncan s multifunctional method in the random of 5 percent.

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 were no significant differences between obtained means. Means were analyzed using Duncan's multiple range tests.

III. RESULT AND DISCUSSION

According to Table II, adding probiotic to the diet of the broilers at different ages and to the different parts of the small intestine had different influences on the activity of Sucrase enzyme. The activity of Sucrase enzyme demonstrated a significant increase only at the age of 21 days in 1% segment and age of 42 days demonstrated a significant increase in 70% segment of the small intestine to that of the treatment groups. Probiotic did not have significant effect on sucrase activity in different ages and segments of small intestine.

After sampling of 1%،10% ،30% and 90% of total length of the small intestine in treatment groups, it was showed that comparing to the control group ($P<0.05$), probiotics did not have a significant effect on sucrase activity in different ages and segments of small intestine [8].

TABLE II
COMPARISON OF AVERAGE SUCRAZE 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)	0.0654^{abd}	0.0780^{abcd}	0.0696^{abcd}	0.0773^{abcd}	0.0684^{abcd}	0.0789^{abcd}
	Protoxin(b)	0.0797^{abcd}	0.0744^{abcd}	0.0719^{abcd}	0.0765^{abcd}	0.0912^{abcd}	0.0986^{abcd}
	Bioplus2b (c)	0.0963^{bcd}	0.0767^{abcd}	0.0771^{abcd}	0.0747^{abcd}	0.0753^{abcd}	0.0761^{abcd}
	biosaf (d)	0.0922^{abcd}	0.0796^{abcd}	0.0793^{abcd}	0.0752^{abcd}	0.0885^{abcd}	0.0836^{abcd}
42	Control (a)	$0,0598^{abcd}$	0.0524^{abcd}	0.0536^{abcd}	0.0518^{abcd}	0.0425^{acd}	0.0637^{abcd}
	Protoxin(b)	0.0781^{abcd}	0.0671^{abcd}	0.0581^{abcd}	0.0480^{abcd}	0.0720^{bcd}	0.0781^{abcd}
	Bioplus2b (c)	0.0790^{abcd}	0.0460^{abcd}	0.0656^{abcd}	0.0359^{abcd}	0.0528^{abcd}	0.0634^{abcd}
	Biosaf (d)	0.0856^{abcd}	0.0477^{abcd}	0.0596^{abcd}	0.0510^{abcd}	0.0548^{abcd}	0.0549^{abcd}

Bacterial patterns in early life were unstable and chickens are susceptible to environmental pathogens. In fact, the initial population of the bacteria is very important for the host because the bacteria can alter the mucosal cells genetically [15]. These bacteria are likely to stay permanently in intestinal and would determine the type of bacteria in later stages of their life [16]. Lastly, the initial bacteria affect the final composition of the permanent intestinal bacteria [17]. These 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 [18]. Villi are more flat and leaf shape in herbivores, whereas in carnivores birds these villi are tall and finger shape [19] villi are covered with enterocytes which are responsible for absorption of food material [19]. Hampson [20] believed that measurements of the villus height and their shape can give us an indication of the enterocyte numbers [20]. According to Ghiasi *et al.* [8] any change in villus height leads to a change in absorption rate [8],[21]. In another study, using the lactobacillus (L.reuteri, L.salivarius) increased the transport of sugars from the intestine and villi height in comparison to the control group [22]. Intestinal disaccharides are enzymes which are gathered in the head brush borther and glycoprotein and break down the disaccharide into monosaccharide; these monosaccharaides are absorbed through Intestinal cells [23]. Nunez et al (1996) showed that disaccharides activity varies in different intestinal parts, so that the activity of disaccharides in the duodenum part is minimum and is maximum in ileum and jejunum parts which is similar to mammals [24]. According to Jones et al. [25] and Burrin [26], the activity of isomaltase and sucrase enzymes at the age of 16 and 17 days of fetal has been reported and their activity increases in the 19 days, but there is no sign of digestion of the carbohydrates before hatching [2]. Two weeks after the hatching, the activity of Sucrase and multase enzymes increases about 2 to 4 times and would then become constant. Their activity is influenced by the diet. Traber[27] and would increase on a starch diet [25], [27] , [28]. Semenza , compared the sucrase and maltase enzymes activity of mammals and chicks, and found that the activity of maltase and sucrase enzymes for poultry is lower compared to that of the mammals [23]. The last stage of carbohydrate digestion occurs on the surface of intestinal mucosa cells. After contact with the intestinal mucosa, it will result in production of carbohydrates and their transfer into intestinal epithelial cells. The enzymes responsible for this step are not available freely in the intestinal tract; they are rather available as integral membrane protein in the plasma membrane of intestinal cells. These enzymes are exposed to microvilli number of cells in large intestinal mucosa which is named the brush borther head. These enzymes are maltase and sucrase [29]. 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 [30], [31].

IV. Conclusion

Adding probiotics to the diet showed no significant effect on increasing sucrase. In other words, adding probiotics to broiler diets had a significant effect on the activity of the sucrase enzyme at the end of the third week in the 1% segment and at the end of the sixth week in the 70% segment of the small intestine.Generally, the sucrase enzyme level in the initial and final segments of the small intestine is low and probiotic has no effect on the absorption of carbohydrates.

Finally, the no great difference was observed between the activity of sucrase enzyme of the experimental and control groups.

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