Effects of Probiotic on the Intestinal Morphology with Special Reference to the Growth of Broilers

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Summary: The probiotic (Protexin)® increases the growth rate in broilers. It must interfere with the intestinal cell morphology and absorption. The intestinal epithelium is one of the most rapidly renewed tissues in the body and is renewed by a process of continuous cell division. This study was carried out with an aim to establish a link between the use of probiotic doses, growth rate, and intestinal cell proliferation by measuring the length and weight of the intestine and intestinal crypt cell proliferation (CCP) of broiler chicks. The results revealed significant increase in intestinal CCP but no effect was observed on the intestinal weight and length. The increase in CCP has also no significant influence towards growth factor. The increased weight gain in this study is associated with more feed consumption which is observed with Protexin® dose 1.0 g / 10 kg of feed. Furthermore, feed consumption reduced beyond this dose may lead to reduced weight gain.

Introduction

Probiotic (Greek word meaning "for life") [1] can be defined as a live microbial feed supplements, which beneficially affects the host animal by improving its intestinal balance [2]. Review of literature indicates that probiotics have effect on the main physiological functions of the gastrointestinal tract, such as digestion, absorption and propulsion [3-6]**.** Probiotics have been used by many researchers in the feeds of farm animals such as poultry birds for improved health and growth rate [7, 8], improved feed utilization, increased resistance to infections, and enhanced eggs production [9]. The use of antibiotics for growth stimulation affects gut microflora, resulting in the reduction of resistance to infection caused by certain bacteria [10]. Therefore, Protexin® helps and repairs the deficiencies in the gut flora [1]. Disturbances of the natural microflora can increase susceptibility to infection but Protexin® addition can restore microflora and increase resistance to infection [11, 12]. Reported mechanisms for probiotics action include (i) direct antibacterial effect, (ii) by competition for nutrients [10], (iii) by stimulation of immunity [13], and (iv) by competing for adhesion receptors [14]. The use of probiotics in the commercial poultry industry is increasing day by day. Therefore, in this study different doses of Protexin® added in broilers rations will either increase intestinal weight and length or intestinal crypt cell. Since intestine is the primary site in the body responsible for food digestion, absorption, and propulsion, any significant alteration in the morphology of chicken's intestine with Protexin® may possibly contribute for the best performance of broilers.

Results and Discussion

The data on the length and weight of intestine is presented in the Table-1. The mean intestinal length of the chickens was not significantly different among various groups. Similarly no significant difference was observed in the mean weight gain among different groups.

Table-1: Mean intestinal length, and weight of chicks fed various doses of probiotic (Protexin).

Groups with doses of Protexin (g/ 10 kg)	Length of Intestine (cm)	Weight of Intestine (g)
Group-A (0.00)	161.75 ± 3.79	63.87 ± 3.34
Group-B (0.5)	159.88 ± 4.18	61.52 ± 1.89
Group-C (1.0)	167.13 ± 4.47	66.60 ± 2.95
Group-D (1.5)	152.00 ± 4.70	62.57 ± 3.30

The data on the chicken's intestinal cells proliferation showed interesting dose dependent results as shown in Table-2. It was observed that the length of the intestine of 10%, 50% and 90% in-group B and C was significantly different at the level of $P < 0.001$. However, no significant difference was recorded in group C and D when compared with 10%, 50% and 90% intestinal length.

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Table-2: Effect of different doses of probiotics (Protexin) on the crypt cell proliferation at the different sites of intestine by metaphase arrest techniques using the vincristine sulphate.

Groups with doses of Protexin (g/ 10 kg)	Cells / Crypt / Hour		
	10% Intestinal length	50% Intestinal length	90% Intestinal length
Group-A (0.0)	12.70 ± 0.41 [a]	12.56 ± 0.32 [a]	13.01 ± 0.37 [a]
Group-B (0.5)	14.98 ± 0.47 [b]	15.20 ± 0.34 [b]	15.09 ± 0.33 [b]
Group-C (1.0)	20.40 ± 0.26 [c]	20.52 ± 0.32 [c]	20.65 ± 0.29 [c]
Group-D (1.5)	20.75 ± 0.22 [c]	20.16 ± 0.21 [c]	20.38 ± 0.30 [c]

Different superscripts are significantly different (P < 0.001).The means with common superscripts are not significantly different.

As far as the consumption of feed is concerned, although among various groups of samples week wise increase in feed consumption took place in all groups but, no significant change was found during first three weeks and in group–B in 4th week and in group-D in 4th and 5th week. In week 4 groups-A and C were not different and similar is the case with groups-B and D (Table-3.) However, groups-A and C were different from groups-B and D with significance value $p < 0.05$. A similar significant difference was observed between groups-B, C, and D. Similarly, the feed intake in the last week *i.e.* 5th week of the experiment was significantly different from one another except groups A and B. The highest feed consumption was recorded in the dose group-C and lowest in the dose group-D.

Table-3: Mean weekly feed consumption of chicks fed various doses of probiotic (Protexin).

Feed Consumed (g)	Groups with doses of Protexin g/ 10 kg feeds			
	Group-A (0.0)	Group-B (0.5)	Group-C (1.0)	Group-D (1.5)
Week 1	1357 ± 14 [a]	1384 ± 19 [a]	1367 ± 19 [a]	1334 ± 20 [a]
Week 2	3266 ± 27 [a]	3276 ± 17 [a]	3246 ± 22 [a]	3299 ± 11 [a]
Week 3	3000 ± 10 [a]	3017 ± 28 [a]	3075 ± 47 [a]	3075 ± 47 [a]
Week 4	8144 ± 12 [ab]	8057 ± 10 [a]	8121 ± 25 [ab]	8052 ± 70 [a]
Week 5	10450 ± 32 [b]	10495 ± 12 [b]	12492 ±50 [c]	10327 ± 36 [a]

The means with different superscripts are significantly (P < 0.05) different from one another. The means with common superscripts are not significantly different.

The live body weights were recorded together on weekly basis although week wise increase was noted in all groups but significant difference was found in last two weeks of the experiment (Table-4). Statistically, no significant change was observed in all groups from first week till third week when compared with controls. However, significant ($P < 0.05$) difference was observed in group-B than group- A in the 4th week of mean live body weight while the difference between group-A and C was not significant. However, group D was statistically different from other groups-A, B, C in 4th week with significance value of $P < 0.05$. In week 5 live body weight in group-B was comparable with controls but was statistically different from other groups-C and D, with a level of significance $P < 0.05$. The highest live body weights were recorded in the dose group-C and the lowest were recorded in the dose group-D for the last week of the experiment.

Table-4: Mean weekly live body weight (g) of chicks fed various doses of probiotic (Protexin).

Body Weight (g)	Groups with doses of Protexin g / 10 kg feeds			
	Group-A (0.0)	Group-B (0.5)	Group-C (1.0)	Group-D (1.5)
Initial Weight	447 ± 10 [a]	443 ± 10 [a]	446 ± 10 [a]	443 ± 10 [a]
Week 1	1284 ± 46 [a]	1308 ± 70 [a]	1325 ± 30 [a]	1282 ± 27 [a]
Week 2	3000 ± 10 [a]	3017 ± 28 [a]	3075 ± 47 [a]	3075 ± 47 [a]
Week 3	5712 ± 25 [a]	6100 ± 40 [a]	6125 ± 85 [a]	5587 ± 24 [a]
Week 4	10650 ± 29 [ab]	11275 ± 28 [b]	10650 ± 23 [ab]	10225 ± 29 [a]
Week 5	13625 ± 49 [b]	14350 ± 50 [b]	14650 ± 23 [c]	13120 ± 48 [a]

The means with different superscripts are significantly (P < 0.05) different from one another. The means with common superscripts are not significantly different.

The data on the feed conversion ratio (FCR) for the different doses and control is presented in Table-5. The feed conversion ratio was significantly lower for the dose group-C of Protexin® from the other groups.

Table-5: Feed Conversion ratio (FCR) of chicks fed various doses of probiotic (Protexin) in the last week *i.e.* 5th week.

Doses of protexin g/ 10 kg feeds	Ration Consumed (g)	Mean Weight-gain (g)	FCR
Group-A (0.0)	10450 ± 32	2975 ± 20	3.51 ± 0.49 [b]
Group-B (0.5)	10495 ± 12	3075 ± 22	3.41 ± 0.67 [b]
Group-C (1.0)	12492 ± 50	4000 ± 00	3.12 ± 0.03 [a]
Group-D (1.5)	10327 ± 36	2895 ± 19	3.56 ± 0.14 [b]

The means with different superscripts are significantly (P < 0.05) different from one another. The means with common superscripts are not significantly different.

This study was based on the hypothesis that the addition of probiotic will alter the intestinal morphology and will either increase the intestinal length and weight or intestinal CCP, which will project more villi numbers and will absorb more food, and therefore, an increased weight gain will be obtained. But this study indicated that the addition of different doses of probiotic has no significant effect on the intestinal length and weight. However, increased CCP was observed at all sites *i.e.* 10 %, 50 % and 90 % of the intestinal length. Thus no direct association or relation of enhanced CCP with any phenomena of growth and weight-gain or FCR can be concluded. The probiotic (Protexin)® contains many species of lactic acid bacteria which contribute to fermentation and acidic digesta, which are responsible for CCP. The bacterial breakdown produces various types of organic acids and is available as a source of energy to the host. The organic acids namely, acetic acid, propionic acid, and butyric acid are the major fatty acids apart from

others produced *via* fermentation in the bowel. Among these fatty acids, butyric acid work as a source of energy for the cells in the intestine and stimulate the CCP. Sonmez and Eren, [15] examined the chickens intestine for villus length, height and width, and crypt cell numbers. In probiotics group, ileal villus height increased, jejunal, and ileal width decreased whereas, cell proliferation increased simultaneously. Probiotic enhances fermentation in chickens, which in turn increases the CCP. The increased intestinal cell proliferation has been studied in relation to fermentation by Al-Dewachi *et al.*, [16] and Ryan *et al.*, [17]. The increased concentration of short chain fatty acids (SCFA) and reduced pH is reported for the elevated CCP [18]. Enhancement of the CCP occurs *via* fermentation because consumption of fermented foods has been associated with enhanced CCP and the causative agents for this are lactic acid bacteria [7]. In other words useful bacterial growth facilitates the fermentative process in all kinds of animals including man. This is of special importance in the ruminants and to some extent in non-ruminants and provides substantial amount of energy to the host *via* fermentation [19]. Khattak *et al.*, [20] reported that enhanced fermentation is associated with enhanced or increased production of SCFA. Results in Table-4 demonstrated that the increased doses of growth promoters than the recommended level have been shown to be associated with decreased feed consumption. These results are in line with previous studies [21-23]. Presumably, the highest dose of probiotics in the feed makes it least palatable for the chicks. Therefore, the feed consumption is reduced as a whole. The weight gain in this study is associated with the addition of probiotic and feed intake. There is also evidence in the literature that feeding a diet containing probiotic increased the body weight of chickens [24, 25] and this increase is partly accounted for increased feed intake [26]. As mentioned earlier, increased probiotics dose was associated with reduced feed consumption [21-23] that may be reflected in the decreased body weight. In Table-5 the feed conversion ratio was significantly lower for the dose group-C of (Protexin)® from the other groups, which is not reported in the literature. In the reported literature it has been shown that the inclusions of probiotic in the rations of chicks do not affect FCR [27-31]. Therefore, it is concluded from this study that the probiotic inclusion in the rations of chicks does not alter the intestinal length and weights, and neither weight gain nor FCR. Probiotic dose 1.0 g/ 10 kg of feed is optimum to improve the intestinal microflora and effectively facilitates the digestion of feed and increases the feed intake and CCP. However, no relationship between CCP and growth performance is observed. The increased weight gain in this study is associated with more feed consumption with probiotic dose 1.0 g/ 10 kg of feed. Furthermore, the pattern is reversed beyond this dose.

Experimental

Protexin a product of Hilton Pharma (Pvt) Ltd, Karachi, was obtained from the local market. The study was conducted in the Department of Poultry Science, Khyber Pakhtunkhwa Agricultural University, Peshawar (Pakistan). Effect of different doses of Protexin on the length and weight of the intestine and intestinal crypt cell proliferation of broiler chicks was studied for 35 days. In this study, five hundred newly hatched chicks (male and female) were obtained from Hi-take Poultry Breeders, Lahore (Pakistan). Out of 500 chicks, 160 male, and female Hubbard chicks were selected having same body weights *i.e.* 35 g. They were randomly divided to 0.0, 0.5, 1.0 and 1.5 g / 10 kg dietary Protexin diet groups of four replicates of 10 birds. All groups were reared in experimental cages on floor facilitated with sawdust. Fresh and clean water was provided at *ad libitum.* All compartments were located in one house and each was provided with feeder, drinker, and proper continuous lighting facilities throughout the experimental period. Strict biosecurity measures were taken to prevent any chances of infection. The Protexin® is a multistrain probiotic, containing 9 strains of beneficial microorganisms which occur naturally in the gut of all healthy birds and animals. These microorganisms include: *Lactobacillus plantarum*, *L. bulgaricus L. acidophilus*, *L. rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopesi*. Each probiotic strain contained within Protexin® has been sourced from the American Tissue Culture Collection (ATCC). Individual strains were grown separately in a fermentation chamber and then freeze dried to form a powder before being blended with other components of Protexin® concentrate in exact concentrations [19]. The commercial feed "National Feed" was procured from the National Feeds Company (Pvt.) Ltd., Lahore (Pakistan). Four experimental rations I, II, III and IV were prepared by feed mixture machine. The ration I was control while ration II, III and IV were added blended with different doses of Protexin® *i.e.* 0.0, 0.5, 1.0, and 1.5 g per 10 kg of feeds. Chicks were weighed on weekly basis during the experiment and weights were recorded. The feed conversion ratio was calculated by dividing mean feed consumed by mean body weight-gain. At the end of the experiment, two chicks from each compartment (8

chicks from each group) were randomly selected and injected vincristine sulphate (1 mg / kg body wt.). Vincristine sulphate (anticarcinogenic) stops cell division at the metaphase stage and cell in this condition can be clearly distinguished. The number of arrested cells per crypt provides a measure of epithelial cells proliferations demonstrated by Goodlad and Wright [32]. Exactly after one hour chicks were slaughtered and the body cavity were opened. The entire small intestine was removed and weighed. The length of intestine was measured and tissue samples were taken from 10%, 50% and 90% of the intestinal length. The pieces of these samples tissue were made and fixed in Carnoy's fluid for 2 hours. The samples were then stored in 70% ethanol. For rehydration and partial hydrolysis, a small piece of fixed tissue (1-2 cm) was placed in 50% alcohol for 5-15 min and then transferred to 25% alcohol for 5-15 min. The sample was then hydrolyzed in 1M HCl for 10 min at 60 $^{\circ}$C, stained in Schiffs reagent for one hour at room temperature, finally removed and rinsed with 45% acetic acid and then placed under the dissecting microscope. When the dissection was completed, a drop of 45% acetic acid was put on top of the sample. An attempt was made to move apart the tissues in order to visualize the intact individual crypt. The arrested metaphase stage in entire crypt was counted under 10 or 40 objective to get the desired magnification. The collected data on various parameters was compiled and analyzed on statistical software. The mean and standard error of the mean along with ANOVA and multiple range tests were carried out using the statgraphics statistical software.

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