INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 19, No. 2, p. 102-110, 2021
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Small intestine morphology evaluation of broiler chicken (*Gallus domesticus*) supplemented with lactic acid bacteria serum (LABS) and added with varying levels of probiotic through drinking water

Niña Mae R. Villar*, Rizza Mae Ceballos

Central Mindanao University, College of Agriculture, Philippines

Article published on August 26, 2021

Key words: Broiler chicken, Lactic acid bacteria serum, Ad libitum feeding, Villi, Duodenum, Jejunum, Ileum villous, Absorptive cells, Goblet cells

Abstract

The study was conducted to evaluate the small intestine morphology of broiler chicken *(Gallus domesticus)*. A total of 60-day-old chicks regardless of sex were randomly distributed into four dietary treatments and replicated three times. Birds were given feed and water ad libitum feeding throughout the rearing period. Treatments were introduced through supplementation in drinking water with the following levels of Lactic Acid Bacteria Serum and commercial probiotics with the following treatments: Treatment 1 = 15mL of LAB serum + 0mL probiotics + 1 liter water (control), Treatment 2 = 15mL of LAB serum + 5mL probiotics + 1 liter water, Treatment 3 = 15mL of LAB serum + 10mL probiotics +1 liter water and Treatment 4 = 15mL of LAB serum + 15mL probiotics +1 liter water. All the data were analyzed using the Analysis of Variance in Complete Randomized Design. Differences among the treatment means showed significant differences were compared using Least Significant Differences. Based on the statistical result, there were highly significant differences observed among treatments ($p>0.01$) on the villous height of the duodenum and jejunum and significant difference in ileum villous height of the three sections of small intestine with those receiving Lactic Acid Bacteria Serum and commercialized probiotic having taller villi. The appearance of absorptive cells and goblet cells did not show significant changes. In conclusion, the results indicated that the Lactic Acid Bacteria Serum and probiotic enhance the performance by improving nutrient metabolizable and digestive tract development.

***Corresponding Author:** Rizza Mae Ceballos ✉ villar@f.ninamae.villar@cmu.edu.ph

Introduction

The poultry industry has become an important economic activity in many countries. In large-scale rearing facilities, where poultry are exposed to stressful conditions, disease-related problems and deterioration of environmental conditions often occur and result in serious economic losses (Nava *et al.*, 2005). According to Charles and duke (1978) the efficiency of poultry depends on the microorganisms which live naturally in its digestive tract. Dietary certain feed additives are products which are incorporated into animal feed create (Charles and Duke, 1978). It is speculated that the benefit derived from probiotics is a results from the organisms organisms growing and contributing some beneficial function in the intestinal tract. According to Zhang *et al.* found some probiotics or synbiotics were effective in increasing the body weight of chickens. Probiotics and competitive exclusion approaches have been used to control endemic and zoonotic agents in poultry.in traditional terms, competitive exclusion in poultry has implied the use of naturally occurring intestinal microorganisms in chicks and poults that were ready to place in brooder house.

Lactic acid has been also extensively utilized as a feed additive to improved feed hygiene and gut milieu (Van de Broek, 2000). Lactic acid as probiotic has the potential to adhere to intestinal epithelial cells (Havener *et al.*, 2009). The use of probiotics as feed supplements to the control of pathogen infection and enhance of immune response in chickens (Pascual *et al.*, 1999; Higgins *et al.*, 2007).

In addition, probiotics many offer other functions, such as maintaining and delete of health and promoting the growth of chickens (Khans *et al.*, 2007). Immediate use of probiotics supplementation at birth is more important and useful in avain species than in other animals (Fuller, 2001).

In recent studies, numerous probiotic strains have been used in poultry production/therefore, the application of probiotics provides a potential alternative strategy to probiotics.

The study aims to evaluate the influence of lactic acid bacteria serum with probiotics on the histological changes in the small intestine of broiler chicken.

Materials and methods

Materials and Equipment

The following materials were used during the conduct of the study: brooding house, rearing pens water troughs, feeding scale, 50-watts bulb, empty sacks, old newspaper, pen, record book, triple beam balance, measuring device (tape measure), triple beam balance, small intestine of the broiler chickens, eviscerating materials (knives), record book, table, plate, pail, and plastic bag.

Experimental Animal

A total of 12 experimental birds regardless of sex from the experiment flock of 60 broiler chicken was subjected for small intestine morphology evaluation supplemented with lactic acid bacteria serum (LABS) added with varying levels of probiotics after 35 days of rearing. This was selected based on the weight nearest to the mean of final weight. These birds view sure as the source of small intestine morphology for evaluation in every treatment and replication.

Experimental Treatment and Design

This study was laid out in a Completely Randomized Design (CRD).

There were four dietary treatments and they are as follows:

Treatment 1 = 1000mL Tap Water + 15mL LAB Serum + 0mL probiotics (control)

Treatment 2 = 1000mL Tap Water + 15mL LAB Serum + 5mL probiotics

Treatment 3 = 1000mL Tap Water + 15ml LAB Serum + 10mL probiotics

Treatment 4 = 1000mL Tap Water +15mL LAB Serum + 15mL probiotics

Preparation Treatments

For the preparation of treatments, the lab serum will be undergoing four major procedures to get the pure stock (ATI-ITCH, 2016).

Preparation of Fermented Rice Wash (FRW)

In this procedure, rice wash (hugas bigas) is the material in producing the lactic acid bacteria. Rice wash is the primary material in to making lactic acid bacteria was fermented at an optimum temperature of 23-25°C in a cool and shaded area with no sunlight.

Approximately three liters of rice wash were collected and placed in a clean container. The container must be big enough to accommodate the rice wash and to occupy 2/3 of the total volume of the container to allow air gap inside. Though lactobacilli are "anaerobic" organisms and can therefore grow on low or even in the absence of oxygen, the air gap will be needed for the fermentation process to proceed.

The fermentation process was completed at approximately seven days. However, on the fourth day, these layers were formed already: floating matter, transparent liquid and dregs. It starts to emit a sour smell unique to lactic acid bacteria. The separated layer of clear liquid contains the lactic acid bacteria (some call this LAB pure stock)

Production of Lactic Acid Bacteria Serum (LABS)

The FRW was inoculated with different types of microbes including lactobacilli. The purification process will take seven days and involve the use of fresh milk to eliminate other microorganisms which allow the increase of lactobacilli population (FRW to milk ratio is 1:10). At 23-25°C, starch, protein, and fats were seen on the surface (called cheese) while yellow liquid (lactic acid bacteria serum, LABS) was noticed at the bottom.

Procedure

The FRW was strained and approximately one liter of it was mixed with 10 liters of milk in a clean container. The container was covered tightly and the date of preparation was properly indicated. After seven days, the resulting light-yellow liquid was harvested and purified

Multiplication of LABS under room Temperature

LABS was mixed with the same amount of molasses added at a 1:1 ratio to keep it under a normal room temperature. The molasses added served as their food that allowed them to survive and continue to multiply.

Utilization of LABS

The "lactic acid bacteria concoction" from LABS with molasses was made using the basic 1:20 dilution ratio and mixed with drinking water.

Care and Management

Before the start of the study, cleaning and disinfecting cages were done to eradicate the presence of any harmful microorganisms in the cage that might harm the experimental animals. The experimental animals were fed adequately, and water was given at all times throughout the study.

Feeding management

The feeding was diluted into two phases wherein during the 1st phase; 14 days of the birds were fed with chick booster mash and on the 2nd phase (15-30 days the birds were fed with broiler starter crumble. The birds were introduced with the treatments at eight days of age, actual amounts of treatment were added until 35 days age of broiler chicken.

Materials and Equipment use for Evaluation

The following materials are used microscope, glass slides, coverslips, weighing scale, bottles, microtome blade, water bath, molder stove, beaker, volumetric flask, stirring rod and spatula and staining reagents will be used. Different percentage (%) levels of alcohol, hematoxylin and eosin stains were also made available.

Experimental Chickens

On day 35, one animal per replication was dressed. During the necropsy, the small intestine was separated and the length was determined.

Portions of the small intestine 10cm from the midpoint of the duodenum, from the midpoint between the point of entry of the bile duct and vitelline diverticulum (jejunum), and midway between Meckel's diverticulum and the ileocecal junction (ileum) from each broiler was cut and subjected for histologic examination.

Histological Processing

After fixation using Bouin's solution for 24 hours, the tissue passed through a series of progressively increasing concentration of alcohol i e. 70% alcohol for an hour, then 80% alcohol for another hour, 90% alcohol fo the next hour and 95% alcohol for the last hour. The tissue was placed in the series of absolute alcohol for 2 hours each, except for the last with 100% alcohol which was soaked overnight.

This was made using xylene 1, 2, and 3 solutions with 40 minute each. The tissues were soaked again in xylene-cedar oil overnight. Soft paraffin was use for 40 minutes each in three series of impregnation. After the tissues were impregnated, these were embedded in hard paraffin. Then cut the four to five sections of the micro tome and staining followed by the use of hematoxylin and eosin stain (Appendix 2).

Histological Examination

Villous height and also the appearance of epithelial absorptive cells and goblet cells at the duodenum, jejunum and ileum were examined and histological changes were noted. Three representative slides from each tissue sample were examined. Five (5) villi were measured and mean villous height were calculated.

Measurement of Villi

Prior to the measurement, the microscope's objective the ocular of the microscope the ocular lens/micrometer combination. A stage micrometer was used in the calibration to come up with the calibration factor of 10. The villi were measured under the scanner microscope with the use of ocular micrometer and LPO objective. Value obtained from the ocular micrometer was multiplied by the calibration factor (10) resulting to the final villous height.

Data Gathered

The measurement of villous height and crypt depth from the three segments (duodenum, jejunum, and ileum) of the small intestines in all treatments were determined and the appearance of epithelial cells and goblet cells were also observed and counted.

Statistical Analysis

The changes in the appearance of the villi is determined using the following parameters:

a. Fully developed – the villi project about 0.5-1mm from the surface of the mucusa (Junquera 1998)
b. Less developed – villi projections is below 0.5mm from the surface of the mucusa
c. Undeveloped – No villi projections from the mucusa

The numerical data were gathered from this study and subjected to the analysis of variance of a Completely Randomized Design (CRD). Observed significant differences among treatments means were further compared using the Least Significant Difference (LSD). A correlation was done with body weight gain and villous height.

Results and discussions

Duodenal Villous Height

Table 2 shows the mean villous height in the small intestine of the broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water. Results show highly significant differences among treatment means in the villous height at the duodenum indicating that the lactic acid bacteria serum and added probiotics has influence on the increase in length of the villous. Both Treatment 1 and Treatment 2, with the mean of 0.6300 and 0.7440mm has highly significant to each other. Furthermore, Treatment 3 and Treatment 4 with the mean of 0.4200 and 0.3740mm respectively, are not significantly different.

The significant increase in villous length (duodenum), indicates an increase in its ability to absorb nutrients. As study conducted by Samanya and Yamauchi, 2002 the longer villi are generally the result of activated cell mitosis and consequently promote growth (Yamauchi et., 2006). The villi of the duodenum of chicken studied were lined by simple columnar epithelium (Aitken, 1958). In addition, Deshpande *et al.*, 2011, states that probiotics have been used as biologically active substance in a large extend of pathologic

conditions, ranging from the antibiotic associated diarrhea, irritable bowel syndrome and lactose intolerance to dental caries.

Sigmon (2014) stated that, the duodenum is where the secretions from the pancreas and gallbladder are added to the stomach contents to neutralize the acid. Enzymes and bile are added in that portion to break down proteins and fat. It receives partially digested food (known as chyme) from the stomach and it plays as a vital role in the chemical digestion of chyme in preparation for absorption in the small intestine.

Table 1. Villous Height (mm) at the duodenum of the small intestine of broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water.

Treatment	Replication					Mean**
	1	2	3	4	5	
1	0.61	0.65	0.63	0.68	0.58	0.6300^{b}
2	0.81	0.76	0.74	0.65	0.76	0.7440^{a}
3	0.63	0.41	0.33	0.29	0.44	0.4200^{c}
4	0.31	0.40	0.38	0.45	0.33	0.3740^{c}

Normal Value = 0.5-1mm (Junguiera, 1998)

CV = 14.71%

** = highly significant

Means with no common letters are significantly different.

Table 2. Villous Height (mm) at the jejunum of the small intestine of broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water.

Treatment	Replication					Mean**
	1	2	3	4	5	
1	0.55	0.62	0.58	0.50	0.49	0.5480^{bc}
2	0.59	0.72	0.64	0.55	0.60	0.6200^{ab}
3	0.64	0.70	0.72	0.67	0.62	0.6700^{a}
4	0.53	0.49	0.50	0.62	0.58	0.5440^{c}

Normal Value = 0.5-1mm (Junguiera, 1998)

CV = 9.14%

** = highly significant

Means with no common letters are significantly different.

Jejunum Villous Height

Table 3 shows the villous height (mm) at the jejunum of the small intestine of broiler chicken supplemented with lactic acid bacteria serum added with varying levels of probiotics through drinking water. Highly significant observed among means villous height in the jejunum with those in Treatment 3 having 0.6700mm and Treatment 4 having 0.5440mm. Furthermore, there is no significant difference in Treatment 1 having 0.5480 and Treatment 2 having 0.6200mm villous height. The presence of increasing numbers of goblet cells, increased in width in some villous were also observed. The jejunum is the second portion of the small intestine, that involves the both the further breakdown of nutrients as well as the beginning of absorption of nutrients. Since absorption is totally dependent on the mechanisms that occur in the intestinal mucosa, the manipulation of probiotics (microbial supplements comprised of specific bacteria or fungi) together with prebiotics (non-digestible ingredients that are beneficial to the host because they selectively stimulate growth and the activity of certain bacteria in the intestine) have been used to improve performance and consequently, the energetic efficiency of the intestine (Hofacre *et al.*, 2003; Pelicano *et al.*, 2004).

In connection, Mescher (2013) stated that mucosa which is best developed in the jejunum, densely covered with villi that project into lumen, thus increasing the surface area for nutrient absorption. This shows that the treatments given of the broiler chicken have the significant effect in increasing the villous height of the jejunum. According to Cera *et al*., 1988 and Pelicano ERL *et al.,* 2005 maximum absorption and digestion capacity is given by a sizeable luminal area with high villi and mature enterocytes, and is essential to animal development. In addition, the effect of lactic acid bacteria serum and probiotic are important because it can increase the enzymes activity in the intestine due to probiotics, thus increasing the digestibility of nutrients in the diet (Fao, 2016).

Ileum Villous Height

Table 4 shows the villous height at the ileum of the small intestine of broiler chicken. Treatment 4 showed significance among the treatment means with 0.7680mm villous height. While the Treatment 1 with the mean of 0.5980, followed by 0.6500 for the treatment 2 and 0.6300 for the treatment 3 are significantly different from each other.

This result obtained from the study, revealed a significantly difference in the villous height of the three proportion of the small intestine. As Yamauchi, (2002), stated that the morphological changes of the intestinal villi in broilers are dependent on the presence of digested nutrients in the small intestine. It expected that in this section the villous are fully developed but in low ranges.

However, it varies on the fasting prior to the necropsy of the tissues that there is slow down of absorption of the nutrient compared to the two proportions of small intestine which are duodenum and jejunum that are both highly significantly difference.

According to DeRouchery (2009), nutrient absorption continues into the final section of the small intestine, the ileum. In connection, Yamauchi (2002), stated hypothesized that stimulation of the ileal absorptive function results in an adaptive compensatory enlargement of the ileum villi. As stated by, Sigmon (2014) the ileum is the last part of the small intestine. Increased digestibility of nutrients in the diet may be due to increased enzyme activity in the intestine due to probiotics According to Fao (2016). The effects of probiotics on villus surface area may change depending on the segment which bacteria colonized. Aggregations of lymphatic nodules, Peyer's patches, are present in the lamina propria and submucosa of the small intestine, especially the ileum.

Table 3. Villous Height (mm) at the ileum of the small intestine of broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water.

Treatment	Replication					Mean*
	1	2	3	4	5	
1	0.64	0.59	0.52	0.65	0.59	0.5980^{b}
2	0.59	0.62	0.66	0.70	0.68	0.6500^{b}
3	0.52	0.58	0.72	0.64	0.70	0.6320^{b}
4	0.68	0.74	0.65	0.98	0.79	0.7680^{a}

Normal Value = 0.5-1mm (Junguiera, 1998)

CV = 12.76%

* = significant

Means with no common letters are significantly different.

Table 4, shows the mean villous heights at the duodenum, jejunum, and ileum of the broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water that the duodenum has the shortest villous heights among the three with the mean of 0.542, compared to jejunum that gains 0.595 and the ileum with 0.662.

However, statistical results showed no significant differences among the means. According to Carlton *et al* (1995) (as cited by Montemayor 2006) the intestinal segments vary its shapes and heights of villi among species. These results were similar to findings reported by Pelicano *et al.* (2005) and Loddi *et al.* (2004) who found, respectively, lower villous heights in the duodenum of control birds compared with birds fed diets containing probiotics and prebiotics. Thus, the higher villi may have resulted from the action of organic acids (those added to the diet with the prebiotics in conjunction with the acids produced by the microbiota), which contributed to a more effective pH reduction in the intestine and consequently, reduced colonization of the intestine by enteropathogenic microorganisms.

Table 4. Summary table in the means villous height (mm) of duodenum, jejunum and ileum of broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water.

Treatment	Doudenum	Jejunum	Ileum	Mean^{ns}
1	0.630	0.548	0.598	0.592
2	0.744	0.620	0.650	0.671
3	0.420	0.670	0.632	0.574
4	0.374	0.544	0.768	0.562
MEAN^{ns}	0.542	0.595	0.662	

Normal Value = 0.5-1mm (Junguiera, 1998)

CV = 20.94%

Ns = not significant

Means with no common letters are significantly different

Changes on the Appearance of Epithelial Absorptive Cells and Goblet cells

The appearances of absorptive cells (Appendix Fig. 13) were apparently normal from the four treatment groups. No significant pathologic changes were observed and no significant differences existed in the appearance of absorptive epithelial cells among treatment group.

According to McDonald *et al*., (2002), the histology of the epithelial cells on the villus apical surface and intestinal villi are influenced by dietary feed components. Intestinal mucosa plays an important role as the site of nutrient absorption in the small intestine (Julendra *et al.,* 2012 and NR. Abdulla *et al.,* 2016). In connection, the small intestinal epithelium is a compound multiple cell system, which determines the growth potential of broiler after hatched (Uni *et al*., 1998).

The development of intestinal morphology and function resulted in the development of chickens (Yamauchi and Tarachai, 2000; Yang *et al*., 2007). As Clevers (2013), stated that the epithelium of the small intestine is organized into large numbers of self-renewing crypt villous units. Differential development of the absorptive epithelium may be responsible for changes in absorption capacity of birds.

The appearance of goblet cells (Appendix Fig. 13) was apparently normal from the four treatment groups. No significant pathologic changes were observed and no significant differences existed in the appearance of goblet cells. The goblet cells (Mescher, 2013) interspersed among the absorptive enterocytes. They secrete glycoprotein mucins that are then hydrated to form mucus, whose main function is to protect and lubricate the lining of the intestine.

Relationship between body weight gain and villous height

The summary of the villous height of the three sections of the small intestine, duodenum, jejunum and ileum, and the body weight gain of broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotic through drinking water were negatively correlated (r=0.019) as presented in Table 5. Statistically the result shows that when there is an increase in villous height the body weight gain is decrease, vice versa.

Considering that there is no significant relationship between these parameters then no sound and conclusion could be made.

Table 5. Correlation analysis of villous height on the body weight gain of broiler chicken supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water.

Parameters	Correlation coefficient	P>f
Villous Height Body Weight Gain	-0.019^{ns}	0.0933

Conclusion and recommendations

A total of 12 broiler chicken which represented each treatment from the growth performance study of broiler chicken supplemented with lactic acid bacteria serum were evaluated after 35 days of feeding trial. There were four treatments replicated three times with one broiler chicken per replication and histological examination of intestinal tract which were conducted at the College of Veterinary Medicine, Central Mindanao University, Musuan, Bukidnon. The specific parameters evaluated include the following: histological intestinal changes of broilers' small intestine supplemented with lactic acid bacteria serum and added with varying levels of probiotics through drinking water.

Results of the means villous height of the three segments of small intestine are summarized as follows. First table is the villous height (mm) of duodenum. Results showed highly significant difference among the four treatments. Means to say that there is a significant increase in villous length of the duodenum that indicates an increase in its ability to absorb nutrients. Second table is the villous height (mm) of jejunum. Results showed highly significant observed among means villous height in Treatment 3 having 0.6200mm and Treatment 4 having 0.5480mm. Means that there is a significant effect in increasing the villous height in the 2 treatments and the effect of lactic acid bacteria serum and probiotic are important because it can increase the enzymes activity in the intestine due to probiotics, thus increasing the digestibility of nutrient in diet (Fao, 2016). And the last table is the villous height (mm) of ileum. The result from the study, revealed a significantly difference on the villous height of the three proportion of the small intestine.

Ileum is the final section small intestine that nutrient absorption continues. It expected in this section the villous are fully develop but in low ranges.

Furthermore, there is an effect of fasting the birds prior to the necropsy of the tissues that there is slow down of absorption of the nutrient in the gastrointestinal tract compared to the two segments of small intestine which are duodenum and jejunum. However, the application of lactic acid bacteria serum and probiotics in broiler chicken clearly indicates that the two probiotics have great potential as alternatives to feed antibiotics and also could be good supplement in increasing the absorption rate of broiler chicken.

It is recommended that the further studies at higher inclusion level of Lactic Acid Bacteria Serum and Probiotic conducted research will require improved understanding of host intestinal physiology, its relationship with intestinal microbes and the mechanism by which these bacteria influence the immune system. In addition, for further study before, during and after the feeding to know the different changes of the villous height and if there is an effect in fasting prior to the necropsy.

Acknowledgement

I want to thank our almighty father for everything, to Central Mindanao University, for conducting my study at the Department of Animal Science and using the facilities to achieve our study, and to the college of veterinary medicine for the guidance and histological examinations of my samples.

References

Abdulla NR, Loh TC, Akit H, Sazili AQ, Foo HL. 2016. Effect of oil on growth performance and gut morphology. South African J. Animal Science **46(No.1)**

Austic RE. 2005. Poultry Production. Philadelphia PA. http://epa.gov/101poultrysystem.Access:January 2017.

Charles OW, Duke S. 1978. The response of laying hems to dietary fermentation products and probiotic-antibiotic combinations. Poult. Sci **57,** 1125. (Abstract).

Fioramonti J, Theodorou V, Bueno L. 2003. Probiotics: What are they? What are their effects on gut physiology? Best Pract. Res. Clin. Gastroenterol **17,** 711-724.

Fritts Ca, Kersey Jh Motl Ma, Kroger Ec, Yan F, Si J. 2000. *Bacillus subtilis* C-3102 (Calsporin) improves live performance and microbiological status of broiler chickens. J Appl Poult Res **9,** 149-55.

Fuller R. 2001. The chicken gut microflora and probiotic supplements. J. Poult. Sci **38,** 189-196.

Green AA, Sainsburry DWB. 2001. The role of probiotics an producing quality poultry products. XV. European Symposium on the quality of poutry meat 9-12 September 2001 kusadasi/ Turkey 245-251.

Khan P, Rothwell EE, Galyov P, Barrow A, Burnside J, Wigley P. 2000. Differential cytokine expression in avain cells in response to invasion by *Salmonella Typhimuruim, Salmonella Enteritidis,* and Salmonella Galiinarum, Microbiology **146,** 3217-3226.

Kleyn R, Chrystal P. 2008. Feeding the young broiler chicken in practice: a review 2008. 23rd World's Poultry Congress. Brisbane, Australia.

Maneewan B, Yamauchi K. 2005. Recovery of duodenal villi and cells in chicken refed protein, carbohydrates and fat Brit. Poultry Sci 46 415 423 [Taylor & Francis Online], [Web of Science®],, [Google Scholar]).

May JD, Deaton JW. 1989. Digestive tract clearance of broilers cooped or deprived of water. Poultry Sci **68,** 627-630.

May JD, Lott BD, Deaton JW. 1990. The effect of light and environmental temperature on broiler digestive tract contents after feed withdrawal. Poultry. Sci **69,** 1681-1684.

Nava GM, Bielke LR, Callaway TR, Castañeda MP. 2005. Probiotic alternatives to reduce gastrointestinal infections: The poultry experience. Animal Health Res. Rev. **6,**105-118.

Nether, wood T, Gilbert HJ, Parker DS, O'donnell AG. 2005. Probiotics shown to change bacterial community structure in the avain gastrointestinal tract. J Appl Environ Microbial **65,** 5134-8.

Pascual M, Hugas JI, Badiola J, Monfort M, Garriga M. 1999. Lactobacillus salivarius CTC2197 prevents Salmonella Enteriditis colonization in chickens. Appl. Environ. Microbial **65,** 4981-4986.

Smith MW, Mitchell MA, Peacock MA. 2007. Effects of genetic selection on growth rate and intestinal structure in the domestic fowl (Gallus domesticus). Comparative Biochemistry Physiology **97A,** 57-63.

Uni Z, Ganot S, Sklan D. 1998a. Post-hatch development of muscosal function in the broiler small intestine. Poultry Science **77,** 75-82.

Uni Z, Noy Y, Sklan D. 1996. Development parameters of the small intestine in heavy and light strain chicks pre and post-hatch. British Poultry Science **36,** 63-71.

YamauchI K, Samanya M, Seki K, Ijiri N, Thongwitaya N. 2006. Influence of dietary sesame meal level on histological alterations of the intestinal mucusa and growth performance of chickens. J. Appl. Poult. Res **15,** 266 273.

Yamauchi K. 2002. Review on chicken intestinal villus histological alternations related with intestinal function J. Poultry Sci 39 229 242 [Cressref],, [Google Scholar]