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Evaluation of some biochemical parameters of serum and intestinal histopathology in broilers feeding with seed pellet and mash

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Key words: Pellet feed, mash feed, intestine histopathology, alkaline phosphatase, total serum protein.

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Abstract

Improving production parameters in broiler poultry industry is the most important objectives. The most important strategies to achieve better performance, is improving formulations and physical form of feed (pellets, Mash). In this study, 10 broiler farms that were fed with pellet and mash feed selected. Vaccination programs, the condition and quality of day old chickens were similar in those farms. According to the nutritional needs of broiler chickens at various ages formulated feed was prepared and then in 5 farms mash and in another 5 farms pellet feed was used. Losses at the farm, located about autopsy and histopathology slides were taken from the intestine. Also at the end of rearing period, blood samples were taken from 15 chickens in each farm and total protein and alkaline phosphatase were monitored. The results showed that serum alkaline phosphatase levels in pellet group were increased significantly in comparison to mash group ($p < 0.05$), but the total serum protein was not different between groups. In histopathological evaluation it was revealed that hyperemia, hemorrhage and edema was not seen in mash group, But in pellet fed group edema and necrosis along with heterophil accumulation and coagulative necrosis was seen and also there was slump of mucus layer into lumen.

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Introduction

Today, with the growing of poultry industry, especially broiler, breeders are trying to improve the various factors affecting the growth rate during the growing period to achieve maximum weight gain and feed conversion ratio at least. Improved production parameters of broiler poultry are one of the main goals of poultry industry in the world. The main strategies to achieve better performance are improving formulations and the quality of the diet.

Research has shown that pellet feed compared to mash feed on the growth of broiler chickens is effective. Also, when using the pellet feed, choice is taken away from the bird and feed intake compared to mash per unit of time increases.

Researchers with the study of the growth rate and carcass composition of broilers that fed with pellet and mash diets from 21 to 42 days showed that increasing of body weight rate with the use of pallet diet is higher than mash diet (Munt *et al.*, 1995). With using of pelleted feed, body weight increases and almost half of the increase in weight is achieved due to the increased concentration of pelleted rations (Allred *et al.*, 1957). Another important factor that is affective in weight gain of poultry with following the consumption of pelleted rations is chemical changes as a result of heat and pressure generated during the pellet process. Researchers mentioned that the increasing of weight because of using pellet feed compared to mash feed is of increase of nitrogen and metabolizable energy of pellet feeds (Hussar and Robblee, 1962).

On the other hand health of grain is the very important factor in the growth of the bird's and without the health of feed, there is possibility of outbreak of many problems such as toxicity. So in choosing the type of feed especially pellet, very important point is the safety of that. Otherwise contamination in pellet feed, flock poisoning, particularly with severe enteritis, which will in turn lead to reduced performance of the herd. In poisoning with pellet, bacterium factors and mycotoxins are mostly involved. Previous studies have shown that the incidence of chronic bacterial infections, globulin serum levels increased.

Alkaline phosphatase is an enzyme-hydrolyzed (Schussler, 1968), and the use of oil in the diet increases secretion of Alkaline phosphatase of intestinal to serum and this process with surfactant-like particles is done. lipoprotein particles secreted by entrocytes and rich of phosphatidyl choline and alkaline phosphatase are of intestinal origin, but the exact mechanism of this phenomenon is not completely understood (Jyotdeep *et al.*, 2011). Studies have shown that at the top of toothbrushes cytosol of intestinal mucosa of birds and mammals, there is significant activity of alkaline phosphatase (Japundzic *et al.*, 1991), and also alkaline phosphatase activity is indicator of intestinal cell maturation and has a key role in the digestion of fatty acids with long-chain and cholesterol (Schussler, 1968).

The results of studies indicate that increased serum protein indicates increased metabolism, digestibility and absorption of protein in the tissues of chickens fed with diets supplemented with prebiotics (Lotfan *et al.*, 2009). The results of examination of the different levels of prebiotics on production and metabolic performance of broiler chickens showed that the mean serum protein of blood was significantly higher in diets containing prebiotics fermacto to diets without prebiotics (control) (El-Gendi *et al.*, 2000). The researchers also reported that the addition of prebiotics in broiler chickens diets increased the serum protein at all time periods of test (Sirvydis *et al.*, 2006).The results of above studies show that , alkaline phosphatase and total protein of serum have a direct correlation with the health of intestinal tissue and also the presence or absence of enteritis and thus, in the present study with the examination of plasma protein, specific liver-enzymes such as alkaline phosphatase, the effects of different types of feeds (mash or pellet) on health of intestine will be examined.

Also, due to the important advantages of pellet feed

for performance broiler herds, cannot be ignored, Therefore, in the present study in addition to biochemical study with histopathological study of intestines, the effect of using pellet on intestinal tissue's of broiler poultry also will be determined.

Materials and methods

In this study, 10 broiler farms that were fed with a pellet and mash feed were chosen. In order to remove disruptive elements in the process of study, vaccination programs, the condition and quality of farm on one day chickens on all farms in the study were similar. Also according to the nutritional requirements of broiler chickens at various ages, formulated feed and after producing feed in 5 farms mash feed was used and in 5 farms pellet of same feed was used.

Histopathologic Study

In each of the above groups, the respective losses were autopsied and from their intestine slide and histopathological studies were performed. After preparing slides, hematoxylin and eosin (H&E) method of staining were used for staining slides. According to that the symptoms of enteritis were observed in the group fed with pellet, for reliability of the results, samples were obtained up to 5 hours after the destruction of poultry. Also the clinical and autopsy findings associated with other infectious agents were not observed during the study period. Place of taking samples from the ileum area and age clash was 6 days old.

Biochemical parameters

Also at the end of the breeding period from every farm of 15 blood samples were collected for the study of total protein and alkaline phosphatase. To measure total protein Biuret reaction and biochemistry kit were used. To measure alkaline phosphatase the kit which manufactured by biochemistry company was used.

Data Analysis

The obtained data was studied with using the statistical software SPSS version 18.0. About the final weight of the herd, serum total protein levels and serum alkaline phosphatase levels, the independent statistic T-test were used. The data were compared with the 95% probability level and differences between groups on the basis of $p<0.05$ was significant and on the basis of $p<0.01$ was described highly significant.

Results

Based on the results of the study, in poultry that were used mash feed alkaline phosphatase levels was 49.70±1.20 and compared to poultry that was used pellet feed 65.75±4.89 was significantly lower ($p<0.01$). Also, the levels of serum total protein, in poultry that was used mash feed, it was 4.05±0.09 and compared to poultry that was used pellet grain 4.16±0.10 was lower and there was no statistically significant difference ($p>0.05$). Also from the aspect of the average of final weight, in under study groups there was statistically significant difference ($p<0.05$), and in poultry that was used mash feed, the amount of final weight was significantly less.

Table 1. Comparison of the average of the alkaline phosphatase, serum total protein and final weight gain of the two under study groups.

	Mash	Pellet	P value
Alkaline Phosphatase	49.70±1.20	65.75±4.89	0.003
Serum Total Protein	4.05±0.09	4.16±0.10	0.45
Final Body weight	2800±28.86	3000±37.52	0.01

Discussion and conclusion

Feed intake is a major factor in both body weight and feed conversion ratio that affects broiler birds. Since so many factors can influence the feed intake, in most cases, solving the problem of low feed intake is difficult, except in doing that a complete review of

dietary and management practices that apply. It seems that factors such as management and herd health more than other factors, have feeding problems with them. Nutritional factors that affect feed intake among mixed flocks rather than individual stocks are common. In contrast, environment or immunological stressors effects have most effects on diversification feed from one flock to another flock. Any management instructions that reduce stressors factors will improve feed intake. In order to achieve maximum success in improving flocks feed intake, it should be investigated to determine the source of stress or disease challenge (Ferket and Gernat, 2006).

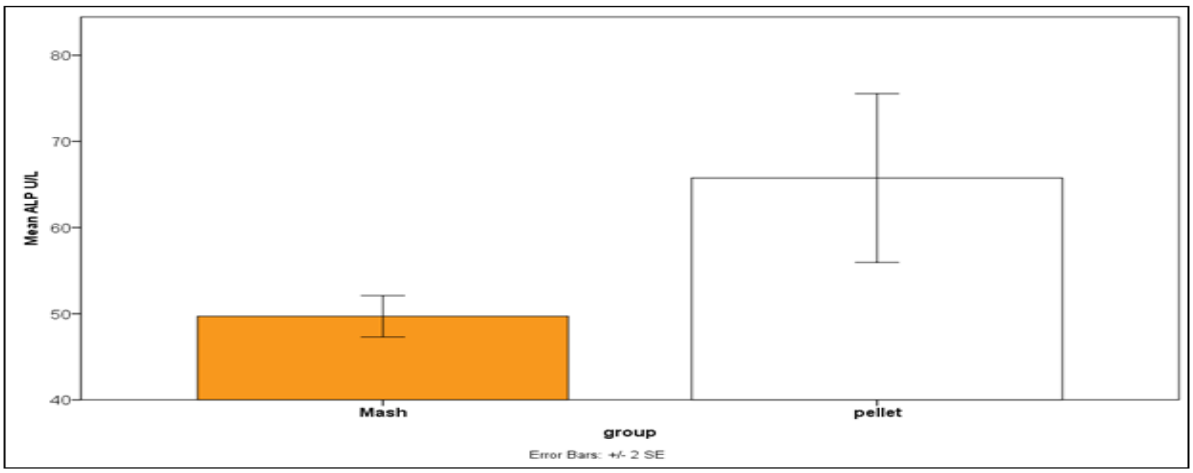

Fig. 1. Graph comparing the mean of alkaline phosphatase levels in the two groups.

The results of a study showed that heating during feed pelleting causing breakdown of disulfide bonds in proteins and thereby denaturation and increasing the effectiveness of enzyme digestion (Morgan *et al.*, 1968).The researchers stated that the reason for weight gain because of using pellet grains compared to mash feed, starch gelatinization, increasing of food digestibility and inactivity of bacteria in food (Rane and Nemade, 1996).

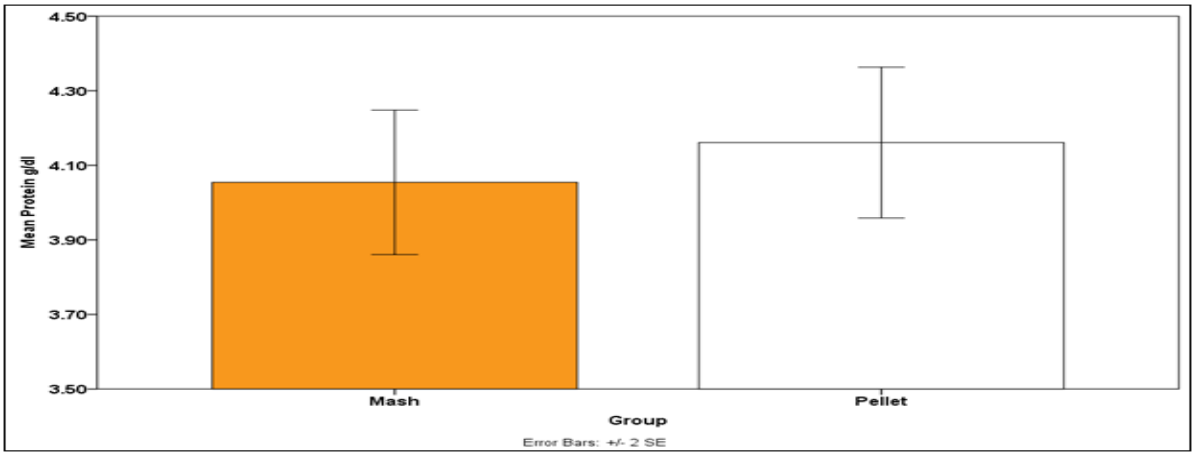

Fig. 2. Graph comparing the mean serum total protein levels in the two groups.

The results of the studies of the researchers showed that effective factors on increasing of the food intake in chickens fed with pellet ration is not well known but perhaps it is because of that chickens prefer pellet rations due to coarser particle size than mash rations (Jensen *et al.*, 1962). Also the results of the study showed that birds that eat mash rations compared to birds that eat pellet rations consume more time eating and it also suggested that food conversion efficiency of broiler chicks fed a pellet ration is better

than chickens fed mash ration, because chickens are fed with pellet rations use less energy (Jensen *et al.*, 1962). Leenstra in a study concluded that with the use of pellet rations than mash rations abdominal fat increases and the reason of increasing of fat was due to higher energy using pellet rations by chickens than mash rations (Leenstra, 1986). By considering expression reveals that the performance of broiler chickens is different than the physical form of the ration.

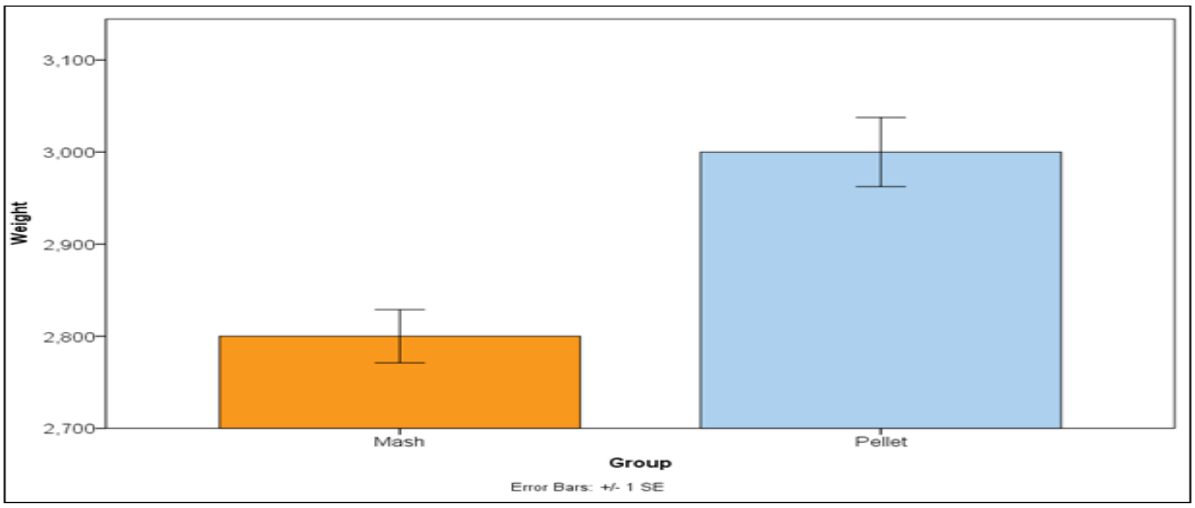

Fig. 3. Graph comparing the average of the amount of final weight of the two groups In the slides for the group fed with pellets mash, there were observed inflammatory response and increasing of the numbers of neutrophils, necrosis, and hemorrhage that the amount of necrosis in pellets is high. In the slides for the group fed with mealy grain, there were observed congestion and little hemorrhage.

Several studies have shown that ration composition affects the amount of alkaline phosphatase, various researchers have studied the serum alkaline phosphatase and intestine after adding all kinds of oily compounds have shown that levels of alkaline phosphatase increase, that is due to the increasing of activities of enzymes of cells with toothbrushes tips, for hydrolysis of fat (Ghiasi-Ghalehkandi *et al.*, 2011). Studies have shown that adding probiotics to the food ration improves the condition of the intestine and increases the amount of alkaline phosphatase (Moosavinasab and Ghiasi, 2011). The results of present study showed that the amount of serum alkaline phosphatase in pellet feed group compared to mash feed group show a significant increase ($p<0.05$), which is consistent with previous studies on the alkaline phosphatase increasing levels.

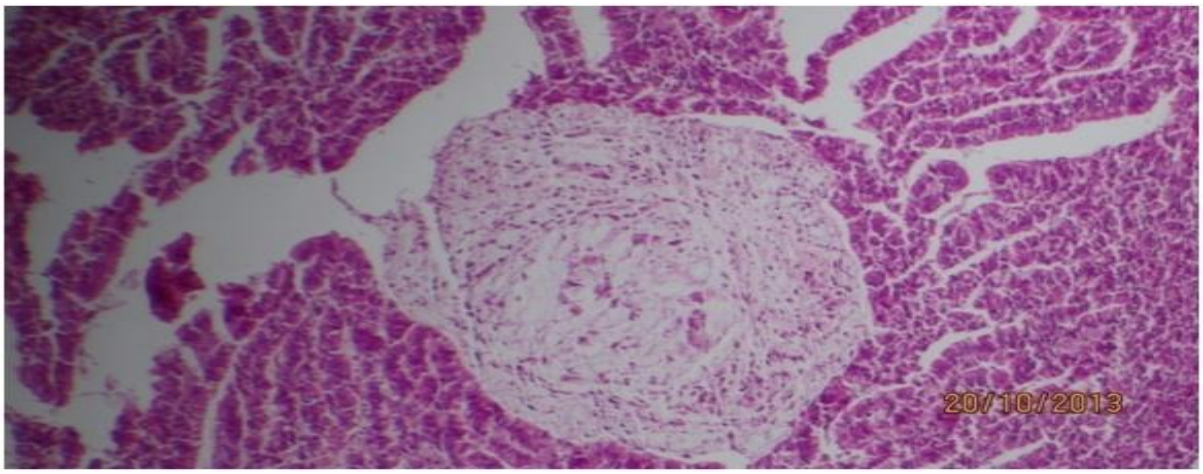

Fig. 1. Intestinal histopathology slides, mash fed group, slight hyperemia under the mucosa.

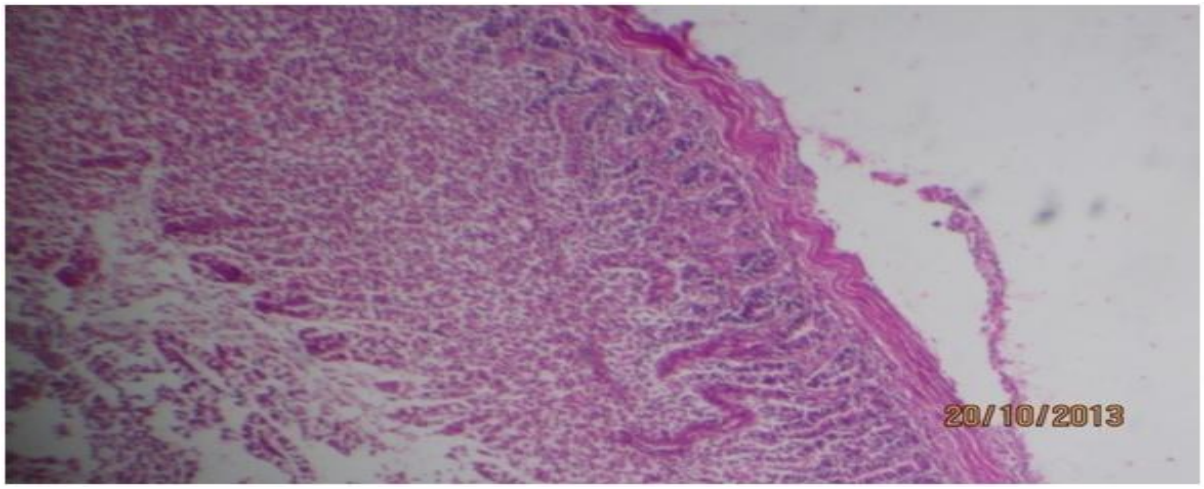

Fig. 2. Intestinal histopathology slides, pellet fed group, congestion and loss (necrosis) villous into the lumen of coagulation necrosis.

Fig. 3. Intestinal histopathology slides, pellet fed group, inflammatory response and increasing of neutrophils.

The results of researches indicate that increasing of serum protein indicates increasing of metabolism, digestibility and absorption of protein in the tissues of chickens fed with rations containing prebiotic (Lotfan *et al.*, 2009). The results of examination of the various levels of prebiotics on production and metabolic performance of broiler chickens showed that the mean of serum protein significantly increases in feeds containing prebiotics fermacto than feeds without prebiotics (control) (El-Gendi *et al.*, 2000).

Alkaline phosphatase is an enzyme-hydrolyzed, which is responsible for taking phosphate group from many types of molecules (nucleotides, proteins, and alkaloids) (Schussler, 1968). The use of oil in the food ration causes of increasing of infiltration of intestinal alkaline phosphatase to serum and this process is done by surfactant-like particles. These lipoprotein particles secreted by entrocytes and they are rich of phosphatidyl choline and alkaline phosphatase with intestinal origin, but the exact mechanism of this phenomenon is not completely understood (Jyotdeep *et al.*, 2011). Alkaline phosphatase includes a group of enzymes that do catalytic activity for the decomposition of phosphate esters and isolating of phosphoric acid molecules and (Moog, 1951). In the toothbrushes part of cytosol of intestinal mucosa of birds and mammals, there is significant activity of alkaline phosphatase (Japundzic *et al.*, 1991). Also studies have shown that activity of alkaline phosphatase is indicator of maturation of intestinal cells and has a key role in the digestion of long-chain fatty acids and cholesterol (Schussler, 1968).

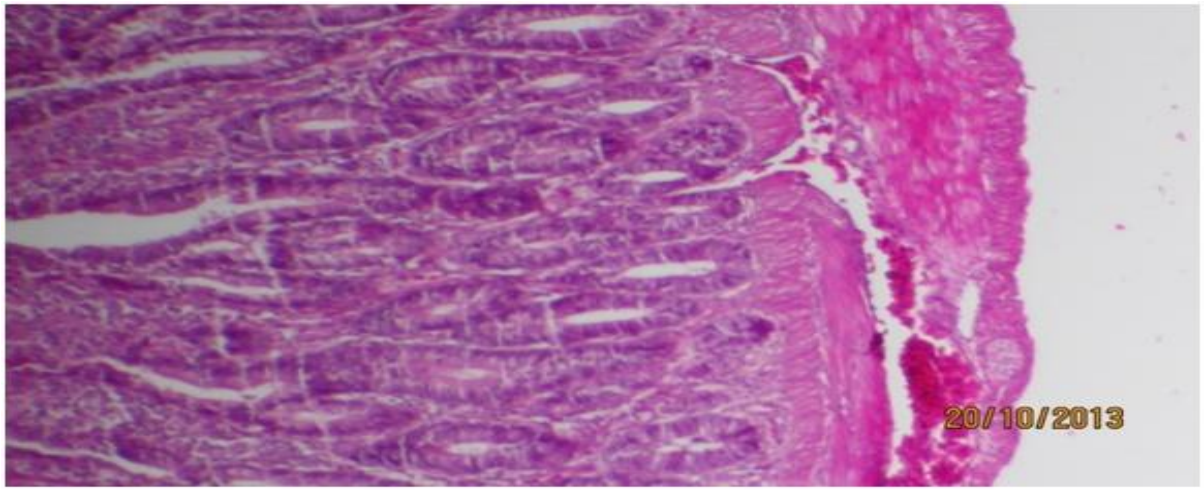

Fig. 4. Intestinal histopathology slides, pellet fed group, congestion under the intestinal mucosa.

Microorganisms are mainly in birds colonized in crop, the cecum and small intestine. Also there are different types of bacteria that colonized in the intestines and eventually lead to changes in the villi of small intestine. Entrocytes covered by villi are responsible for nutrient absorption and every changes of height of villi led to changes in the amount of absorption (Ghiasi *et al.*, 2010- Sklan, 2011).

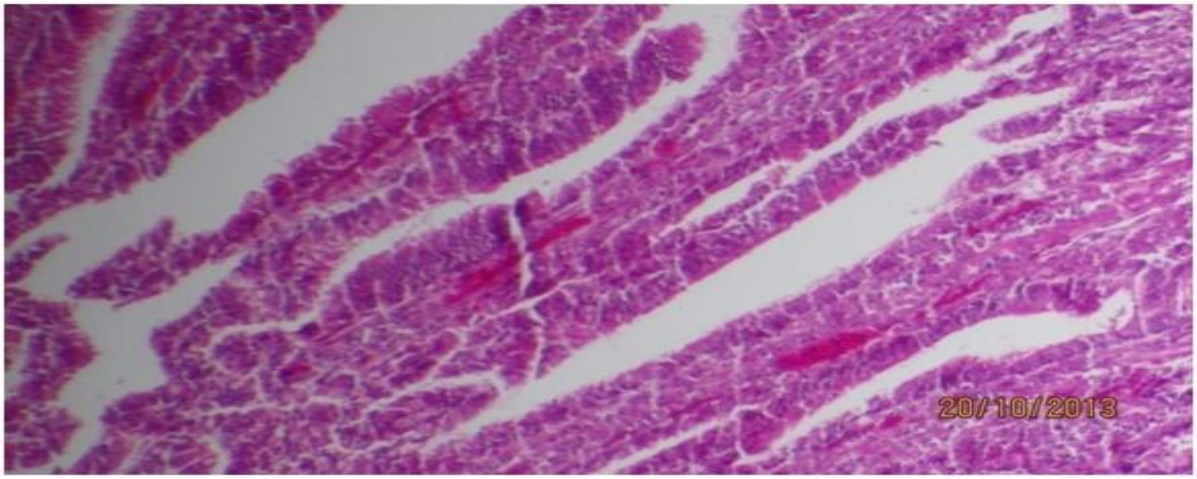

Fig. 5. Intestinal histopathology slides, pellet fed group, bleeding in the intestinal villi.

The researchers also reported that the addition of 0.3 prebiotic to broiler chickens rations cause increasing of serum protein at all period of testing compared with the control group (Sirvydis *et al.*, 2006). These researchers also stated that the change in total protein may indicate the increasing of protein metabolism in the body organs of chickens. The result of above studies show a direct correlation of alkaline phosphatase and serum total protein with health of intestinal tissue and also the presence or absence of enteritis.

In the microscopic examination of the mash and pellet samples, it revealed that in mash samples hyperemia and little hemorrhage were observed and also necrosis and inflammatory reaction were not observed. However, in samples that were fed by pellet, there were observed inflammatory reaction and necrosis that cause the accumulation of neutrophils at that area and there was observed coagulation necrosis. Also in the pellet group, intestinal mucosa poured into the lumen and has a bad condition compared to the group that was fed by mash feed. Also in those samples that were fed by mash feed, there was observed slight hyperemia under intestinal mucosa, but in samples that were fed by pellet, there were observed lot hyperemia under mucosa.

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