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THE EFFECT OF CHITOSAN ON LEAD (Pb) CONTENT IN LIVER AND BONE OF QUAIL EXPOSED TO Pb-ACETATE IN DRINKING WATER

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Abstract

The purpose of this study was to determine the effect of chitosan on the Pb content in the liver and bone of quail exposed to Pb-acetate in drinking water. Quail was kept in experimental cage of Poultry House, meanwhile, slaughtering and sampling were conducted in the Laboratory of Animal Physiology and Biochemistry, Faculty of Animal Husbandry, Padjadjaran University; furthermore, samples were analyzed in the Laboratory of Dairy Cattle, Bogor Agricultural Institute. The Experiment based on an experimental design with five treatments and five replications. Chitosan was given in ration as follows P_0 (0 ppm), P_1 (50 ppm), P_2 (100 ppm), P_3 (150 ppm) and P_4 (200 ppm). Orthogonal polynomial test was used to see the trend of increased or decreased as a result of treatment response. The result of this study showed decreased levels Pb in liver and bone of quail along with increasing doses of chitosan given. The best result obtained at P_4 (200 ppm) with the lowest Pb content in liver and bone of quail.

Key words: *lead, liver, bone, quail, Chitosan.*

INTRODUCTION

Animal products such as meat, egg or milk should be healthy and free from residues of various digestive mainly heavy metals because if the animal products consumed by humans constantly and in large quantities, it will cause health problems and even death for consumers.

Heavy metals such as lead (Pb) are one of the high level of environmental pollutants.

One of the main factors due to the increasing number of industries that use heavy metals pollution that ultimately leads either directly or indirectly to livestock, especially quail.

Direct are effects in the form of pollution through the air, soil and water, whereas the effects indirectly through the form of food and drinking water.

According to Darmono (1995), based on the need for livestock heavy metal, the metal is divided into two essential and non essential. Metals essential needed in the physiological aspect of the animal so that the metal in this group are nutrients that if a deficiency can cause abnormalities of the physiological called mineral deficiencies, while the non-essential is a group of metals that are not useful or not known usefulness in an animal's body, therefore the presence of these elements more than normal can cause poisoning.

Furthermore, it can be said of the main food of the animal species of birds are the seeds that contain high carbohydrates.

This causes metal contamination on the type of grain and metal contamination of drinking water to be the main source of metal toxicity.

One of the leading Indonesian products from the field of fisheries for the purpose of export is shrimp and generally in utilization remains solid waste such as skin, head, and feet.

The solid waste is processed and it can provide additional benefits because it can be used as material for chitosan.

Lead mechanism becomes toxic due to replace Pb-active metal cations such as calcium, zinc and protein. Calmodulin binding four cations eg calcium and lead when replacing four cations calcium deficiency will occur enzim. Along with this enzyme deficiency will hamper plumbum total (delta -ALAD) and when the metal is zinc cation replace its single meal will disrupt the process of blood clotting so there will be severe anemia (Sutrisno, 2006).

Chitosan is a natural polymer that is non-toxic, more environmentally friendly and easily degradable in nature. Chitosan has the

properties of absorbing and clumping well, therefore these compounds can be used as an absorbent material of heavy metals such as Pb. Chitosan has amino (NH_2) relatively more than chitin so that more nucleophilic and alkaline.

Crystallinity of chitosan caused by intermolecular hydrogen bonds is lower than chitin making it easy to apply in some reagents. Chitosan has properties that are not soluble in water and some organic solvents such as dimethylsulfoxide (DMSO), dimethylformamide (DMF), organic alcohol solvent and pyridine; but chitosan is soluble in organic acid / mineral dilute through protonation free amino group on the pH less than 6.5. The good solvent for chitosan is an acid such as formic acid, acetic acid, and the acid glutamate and solubility decreases with increasing molecular weight of chitosan.

Lead levels in the liver and bone quail is a good indicator to indicate exposure, because the levels of Pb in the liver can describe the level of lead in the body. This is because Pb contained in the liver to be detoxified and accumulate in the bone or quail.

The liver is the organ that secretes bile which is channeled into the duodenum which weighs 3% of their body weight. The liver is the defense of life and plays a role in almost every function of the body's metabolism. The liver has a large reserve capacity and network functions to defend the body and the liver also has the ability to regenerate awesome. Liver damage mostly in most cases cell death or illness, it will be replaced with a new liver tissue.

Hepatic function in the body is essentially that as filter toxins, heavy metals that enter through food or drinking water will be filtered in the liver, but the liver also has a threshold in the poison screening. Pb unfiltered most will settle in the liver and in the long term will result in liver function. The series of processes that occur in the liver can result in severe damage to the liver with the result function and structure of the liver cells, which in turn could adversely affect the health and whole organ (Antoine et al, 2008).

Bone formation takes place continuously and can be lengthening and thickening of the bone. Rate of bone formation changes throughout life. Bone formation is influenced by hormonal stimulation, dietary factors, and the amount of stress imposed on a bone, and is the result of the activity of bone-forming cells.

The damage to the liver caused by lead exposure can be detected by screening biochemical and *histopathological* examination of the liver. One of the biochemical examination of the liver that are useful for this purpose is the examination of class transaminase enzyme levels, namely, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT). AST can be found at various places in the body, but more useful as a marker of liver damage and liver, while ALT is more concentrated in the liver.

Quail known as a model animal and a great potential to be developed, is due to the advantages of the properties owned by his quail as small, rounded, and the tail is very short; a relatively short reach sexual maturity at 42 days; egg production can reach 300 eggs per year; ration needs is not too big, ie 14-24 g per head per day and colored feathers brown spots; land needs not be so broad that quail cage can be nested to save the location and maintenance costs are relatively low compared to other poultry accordance smaller body size.

MATERIALS AND METHODS

This research was conducted at the Faculty of Animal Husbandry, Padjadjaran University for 40 days. The material used quail females aged 2 weeks (phase grower) as many as 100 birds, consisting of five (5) treatments that were P_0 (without chitosan), P_1 (50 ppm chitosan), P_2 (100 ppm chitosan), P_3 (150 ppm chitosan), P_4 (200 ppm chitosan) with five replicates and each unit consisted of 4 tail treatment.

Experiment using a completely randomized design (CRD) and the data were analyzed by analysis of variance, significant treatment further continued with *orthogonal polynomial test.*

Variables measured were: Pb content of the liver and bone of quail exposed to acetate Pb in drinking water. Rations used during the experiment were purchased from Poultry Shop PT. Charoen Phokpand Indonesia Tbk with feed ingredients as follows: corn, bran, soybean meal, meat and bone meal, fish meal, coconut cake, broken wheat, canola, calcium, phosphorus, vitamins, trace minerals and anti-oxidants.

RESULTS AND DISCUSSIONS

(1) Effect of Chitosan on the Content of Pb Livers in Quail Exposed to Pb Acetate in Drinking water

Based on Table 1 it shows that there is a decrease of Pb in the livers of quail with the level of administration of chitosan.

The average of the highest concentrations of Pb obtained at P_0 (without administration of chitosan) that is equal to 1,131 and the lowest Pb contents obtained at P_4 is equal to 0.839 ppm (200 ppm chitosan). It shows that chitosan can absorb Pb so it does not accumulate in the liver.

Furthermore, the data were analyzed using analysis of variance and the results is significant ($P<0.05$).

Based on the results of the analysis showed that the chitosan gave effect to the content of Pb in the livers of quail.

Then proceed with the advanced test and orthogonal contrasts was obtained linear equation $y = -0,0014x + 1.1126$ (illustration 1).

Table 1. Effect of Chitosan on the Content of Pb Livers in Quail Exposed to Pb Acetate in Drinking Water

Replication	Treatment (ppm)					Total
	P0	P1	P2	P3	P4	
R1	1,136	1,045	0,957	0,926	0,822	4,886
R2	1,141	1,035	0,945	0,907	0,825	4,853
R3	1,138	1,043	0,952	0,907	0,866	4,906
R4	1,109	0,993	0,950	0,909	0,842	4,803
Total	**4,524**	**4,116**	**3,804**	**3,649**	**3,355**	**19,449**
Average	**1,131**	**1,029**	**0,951**	**0,912**	**0,839**	**4,862**

Notes: P0: Without chitosan; P1: 50 ppm chitosan; P2: 100 ppm chitosan; P3: 150 ppm chitosan; P4: 200 ppm chitosan

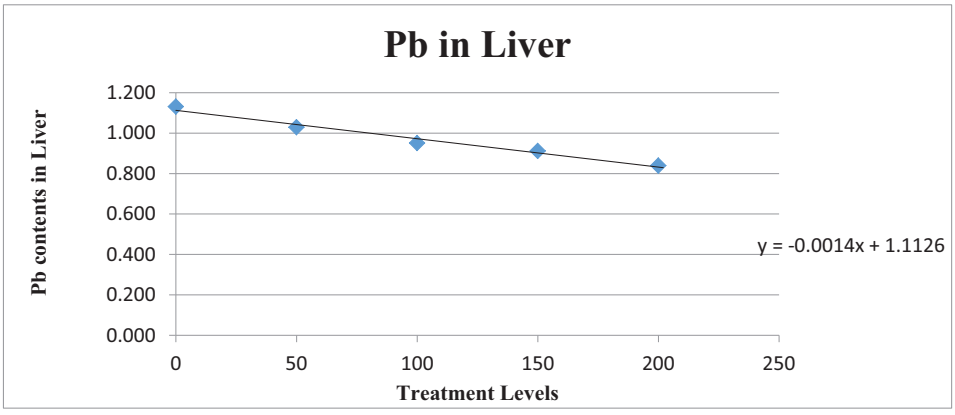

Figure 1. The content of Pb in the Liver Quail

Based on the chart above shows that decreasing levels of Pb in the liver caused by the level of giving chitosan. Chitosan concentration that could absorb Pb maximally obtained at a concentration of 200 ppm. On average 10-30% Pb inhaled absorbed through the lungs, and about 5-10% of ingested absorbed through the gastrointestinal tract (Palar, 1994). Furthermore, absorbed lead is transported by the blood to the organs as much as 95% Pb in the blood bound by erythrocytes. Most Pb plasma in a form that can diffuse and estimated in balance with pool Pb body which is divided into two, namely to soft tissue (bone marrow,

nervous system, kidneys, liver) and to the hard tissues bones, nails, hair, teeth.

Pb is not absorbed in the digestive tract and blood would bring his whole body and can accumulate in other organs including the liver. Alveolar cleaning function is carried particles to mucociliary escalators, through the layer of lung tissue and then towards the lymph nodes and blood stream. As many as 30-40% Pb in absorption through the respiratory tract will get into the bloodstream.

Pb excretion in several ways, the most important is through the kidneys and gastrointestinal tract. Pb excretion via urine as much as 75-80%, through the feces of 15% and more through the bile, sweat, hair, and nails (Palar, 1994). Pb excretion via the gastrointestinal tract is affected by the active and passive channel salivary glands, pancreas and other glands in the intestinal wall, the regeneration of epithelial cells and biliary excretion. Meawhile, the excretion process Pb through the kidneys is through glomerulus filtration. Pb in urine reflects recent exposure to Pb examination of urine used for occupational exposure (Goldstein & Kippen, 1994).

With the availability of chitosan can be expected that Pb can be absorbed by chitosan and can be excreted through urine and feces, because if it accumulates in the liver can cause liver damage. In general, Pb excretion is running very slow. Lead in the blood half-life of approximately 25 days, at 40 days, whiles the soft tissues in the bones of 25 years (Nordberg, 1998).

Hepatic function in the body is essentially as a toxin filter, heavy metals that enter through food or drink into the body will be screened at the liver, but the liver also has a threshold in the poison screening. Pb unfiltered most will settle in the liver and in the long term will result in liver function. The series of processes that occur in the liver can result in severe damage to the liver with the result function and structure of the liver cells, which in turn could adversely affect the health and whole organ (Antoine et al, 2008).

According to research Alifia and Djawad (2000) mentions that the milkfish (Chanos Chanos Forskall) exposed to metallic lead caused liver fatty degeneration. Fatty degeneration is characterized by the appearance of histological vacuole-vacuole. State of the network that has been damaged is due to liver has been exposed to toxic substances (lead). If the toxic substances that enter the body are relatively small or less and liver detoxification function well, then there is no damage, but if the toxic substances that enter in large numbers, the function of detoxification will be damaged (Lu, 1995).

(2) Effect of Chitosan on the Content of Pb Bones in Quail Exposed to Pb Acetate in Drinking Water

Table 2 shows that Pb decrease in bone of quail with the level of administration of chitosan. The average of the highest concentrations of Pb obtained at P_0 (without the administration of chitosan) that is equal to 0.788 ppm and averaging the lowest Pb contents obtained at P_4 is equal to 0.707 ppm (200 ppm chitosan). It shows that chitosan can absorb Pb so it does not accumulate excess bone.

Table 2. Effect of Chitosan on the Content of Pb Bones in Quail Exposed to Pb Acetate in Drinking Water

Repeat	Treatment					Total
	P0	P1	P2	P3	P4	
R1	0,810	0,746	0,734	0,722	0,707	3,719
R2	0,776	0,752	0,737	0,732	0,707	3,704
R3	0,794	0,741	0,731	0,730	0,707	3,703
R4	0,772	0,750	0,739	0,725	0,707	3,693
Total	**3,152**	**2,990**	**2,940**	**2,909**	**2,828**	**14,820**
Rata-rata	**0,788**	**0,747**	**0,735**	**0,727**	**0,707**	**3,705**

Notes: P0: Without chitosan; P1: 50 ppm chitosan; P2: 100 ppm chitosan; P3: 150 ppm chitosan; P4: 200 ppm chitosan

Furthermore, the data were analyzed using analysis of variance and the results showed a significant (P<0.05). This suggests that the chitosan acts as a heavy metal adsorbent.

Then conducted further tests orthogonal contrast with the results of the linear equation y = -0,0004x + 0.7772 (illustration 2)

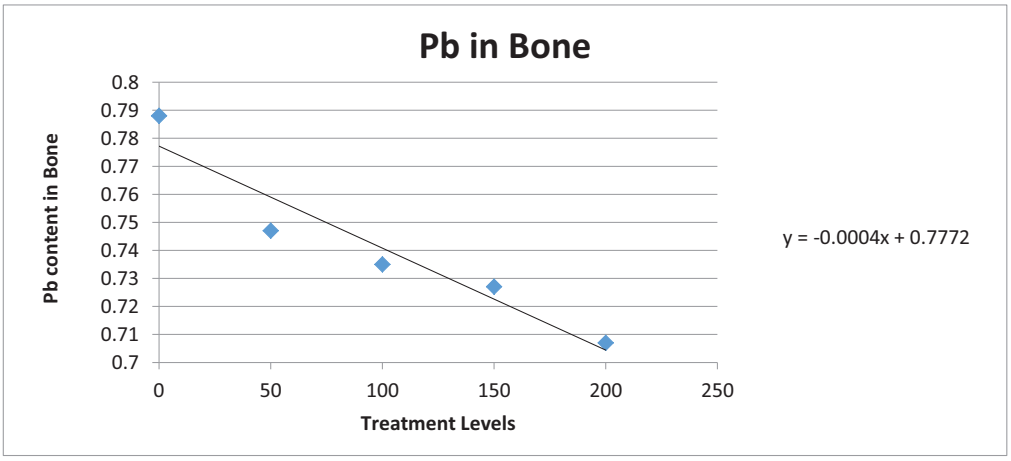

Figure 2. The content of Pb in bone quail

Based on the figure 2 can be seen that Pb accumulation in the bones can be decreased because it is absorbed by chitosan. Best chitosan concentration contained in P_4 (200 ppm). Provision of chitosan can bind heavy metals which accumulate in the body. In the body of the animal, the metal lead gets into the small intestine and is absorbed by the blood which then binds to blood proteins which are then distributed to all body tissues. Highest metal accumulation usually in the liver, soft tissue such as bone and teeth, and is excreted by the kidney (Darmono, 2001). Research Hasan and Seth (1981), reported that the administration of lead in mice can reduce the activity of enzyme δ -ALAD so that Pb can accumulate in bone marrow and become toxic.

In the bone formation process erythrocyte cells, red blood cells is a complex form chelate formed by metal Fe (iron) with a group of hemoglobin synthesis of the complex involves two enzymes, ie enzymes-ALAD (Amino levulinic acid Dehidrase) or amino acids levulinat dehidrase and ferrokhelatase enzyme. ALAD Enzyme is an enzyme type cytoplasm. This enzyme will react actively at an early stage during the synthesis and red blood cell circulation takes place. Hematopoietic system is very sensitive to the effects of Pb. Pb hematopoetic effect is to inhibit the majority of enzymes involved in heme biosynthesis. Among the enzymes involved in heme, an enzyme δ-aminolevulinik acid dehydrogenase (δ -ALAD) and ferrochelatase including enzymes are most susceptible to the inhibitory effect of Pb (Goldstein and Kipen, 1994).

Chitosan can absorb heavy metals based on the nature non-toxin and easily degraded. Chitosan is a polysaccharide amine process results deacetylation of chitin. Polycationic nature of chitosan compounds can be applied in various fields such as metal adsorbent, absorbent dye textiles, materials for cosmetics and antibacterial agents (Bhuvana, 2006).

Effect of chitosan can absorb Pb in bone because chitosan has amino (NH_2) relatively more than chitin so that more nucleophilic and alkaline. This makes the alkaline properties of chitosan: Soluble in dilute acid medium form a viscous solution so that it can be used in the manufacture of the gel. In some variations of configurations such as grain, membranes, coatings capsules, fibers and sponges.

Forming insoluble complexes with water with poly-anion can also be used to manufacture the gel granules, capsules and membranes. Can be used as chelating heavy metal ions in which the gel provides a production system to the effects of destruction of the ion (Meriaty, 2002).

CONCLUSIONS

Based on the results of research and discussion, it can be concluded that the administration of chitosan in the diet lower levels of Pb in liver and bone of quail. Best dose obtained at a dose of 200 ppm (P_4). Administration of chitosan is able to absorb Pb contents in liver and bone quail.

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