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Characterization of proteoglycan products of naturation of chitosan extraction liquid waste with sulfate ions

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Abstract

The process of naturation of chitosan extraction liquid waste into proteoglycans with various types of binders is an effort to make new materials / materials that are expected to provide benefits to increase livestock productivity. The new material (proteoglycan) is formed through a reaction mechanism that is highly dependent on the original component as well as additional components that function as receptors (inhibitors) or activators (triggers) for the formation / occurrence of molecular adhesion (chelating) to a core chain. In addition, the process of naturation of liquid waste is also an effort to manage or handle the environment from waste which if not handled can have a negative impact on the environment. This can happen because the denaturation of protein components or molecules contained in liquid waste becomes pollution due to the decay process.

The purpose of the study was to obtain the character of proteoglycan quality as a result of the addition of various types of sulphate ion binders through measuring the digestibility and hematologic values of blood in broiler chickens. The study used experimental methods in the laboratory. The experimental design used was a Complete Randomized Design (6 X 4), with six treatments (3 types of chemical product binders and 3 types of biological product binders), which were repeated four times. The effect of the treatment was statistically tested by fingerprint analysis, and the difference between treatments was tested with the Duncan Multiple Distance Test.

The research was carried out in two stages, namely the preparation stage (making proteoglycans with the addition of three types of sulphate ion binders), and the product quality testing stage. Product testing through measurement of digestibility (dry matter and protein) and hematologic (number of erythrocytes, leukocytes, and haematocrits) values of broiler blood.

The results showed that liquid waste extracting chitosan from shrimp shells (both through chemical and biological processes) can be used as a source of making proteoglycans and can be used as a feed supplement in broiler chicken rations. Quality characteristics of proteoglycan products: good quality Proteoglycans of chemical and biological processes with potassium sulphate binders; Moderate quality chemical and biological process proteoglycans with ammonium sulphate binders, and biological process proteoglycans with sodium hydro sulphate binders; and Poor-quality Proteoglycan chemical process with sodium hydro sulphate binder. The number of erythrocytes, leukocytes and haematocrits blood of broiler chickens are normal for all types of products.

Keywords: Chitosan extraction; Binder type; Proteoglycan; Digestibility; Hematologic; Broiler

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1. Introduction

The process of naturation of chitosan extraction liquid waste into proteoglycans with various types of binders is an effort to make new materials / materials that are expected to provide benefits to increase livestock productivity. The new material (proteoglycan) is formed through a reaction mechanism that is highly dependent on the original component as well as additional components that will function as receptors (inhibitors) or *activators* (*triggers*) for the formation / occurrence of molecular attachment (*chelating*) to a core chain. In addition, the process of naturation of liquid waste is also an effort to manage or handle the environment from waste which if not handled will have a negative impact on the environment. This can happen because the denaturation of protein components or molecules contained in liquid waste becomes pollution due to the decay process.

Among the fishery products developed by the government is the development and post-harvest management of the shrimp pond business for export purposes [1]. Waste from these activities is in the form of shrimp shells. Efforts to manage this waste are carried out by utilizing it as raw material for the chitosan industry [2,3]. Chitosan is widely needed in industrial activities, such as the pharmaceutical, textile, and food industries [4,5].

As the demand for chitosan increases, and the chitosan manufacturing industry becomes more prevalent, the greater the volume of liquid waste generated from the chitosan extraction process. Liquid waste from chitosan extraction is rich in dissolved nutrients, including gluco-amino-glycans and uronic acid which have the potential to be forming/constituents of proteoglycans [6]. In addition, chitosan extraction liquid waste is in a biologically inactive condition because it has undergone a chemical or biological denaturation process [7]. If this is allowed, there will be a continuous denaturation that has a negative impact on the environment [8].

In order to overcome this, efforts are made to naturate chitosan extraction liquid waste by adding a binder which is expected to be a binding material for the nutrient substances contained in the waste, and carrying out a polymerization reaction that ends with an esterification reaction to stop the polymerization process / reaction.

This study aims to find and characterize the products of the denaturation process (liquid waste) into proteoglycans with binders containing sulphate ions. and its effect on digestibility, metabolic energy, and blood haematology.

2. Research methods

The study was conducted experimentally in the laboratory with the following stages

- Preparation Product propagation (*scale up*) chitosan extraction both chemically and biologically.
- Making proteoglycans from chitosan extraction materials, the best optimization products were selected based on the results of research in year 1.
- Biological testing of proteoglycan products from three types of sulphate ion binders that have been characterized physically and chemically in year 1, through measurements of the digestibility of dry matter and protein of proteoglycan products, and the hematologic value of blood in broiler chickens [9].

$$\text{Digestibility} = 100\,\% - 100 \left\{ \frac{\%\ \text{Indicators in the ration}}{\%\ \text{Indicators in the faeces}} \text{X} \frac{\%\ \text{nutrients in the faeces}}{\%\ \text{nutrients in the ration}} \right\}$$

- Measured Parameters include
  - The content of dry matter and crude protein of proteoglycan products (%)
  - Lignin content of proteoglycan products (%)
  - Dry matter content and crude protein faeces (%)
  - Content of faecal lignin (%)
  - Number of Erythrocytes (mm/dl)
  - Number of Leukocytes (mm/dl)
  - Haematocrits Value (%)

The experimental design used was a Complete Randomized Design (6 X 4), with six treatments (3 types of chemical product binders and 3 types of biological product binders), which were repeated four times. The effect of the treatment was statistically tested using fingerprint analysis, and the difference in influence between treatments was tested with the Duncan Multiple Distance Test.

3. Results and discussion

3.1. Treatment of Digestibility of Proteoglycan Products in Broiler Chickens

The potential nutritional value of proteoglycan products from shrimp waste extract can be determined by chemical analysis. The actual value is indicated from the missing portion after the food ingredient, absorbed and metabolized [10]. The average ration digestibility value was analysed with a fingerprint and the results showed that the treatment had a real effect (P<0.05) on the ration digestibility value. In order to determine the difference in influence between treatments, a Duncan Multiple Distance Test was carried out, the results of which can be studied in Table 1.

Table 1 Average Digestibility Value of Proteoglycan Products in Each Treatment

Treatment	Digestibility of Dry Matter	Protein digestibility
	..... (%).....	
PgKA	66.17 [d]	65.38 [d]
PgKP	72,30 [a]	73,36 [a]
PgKS	58.60 [a]	56.44 [e]
PgBA	66.12 [d]	67,35 [c]
PgBP	70,38 [b]	71,66 [b]
PgBS	68,41 [c]	67,17 [c]

Note: [a, b, c, d, e] means different superscripts within the same column significantly (P < 0.05); Ket: PgKA = Proteoglycan chemical process with ammonium sulphate binder ; PgKP = Proteoglycan chemical process with potassium sulphate binder ; PgKS = Proteoglycan chemical process with sodium hydro sulphate binder; PgBA = Proteoglycan biological process with ammonium sulphate binder ; PgBP = Proteoglycan biological process with potassium sulphate binder; PgBS = Proteoglycan biological process with sodium hydro sulphate binder

Table 1 shows that the dry matter digestibility values in the ammonium sulphate binder treatment ($(NH_4)_2SO_4$) from both chemical and biological extraction results both showed no noticeable difference (P>0.05) but real (P<0.05) were higher than PgKS, and lower than the potassium sulphate ion treatment and sodium sulphate binder from biological extraction. The use of this type of sodium sulphate binder has the lowest digestibility of dry matter. The protein digestibility value in proteoglycan products with the type of ammonium hydrosulphate binder did not differ markedly (P>0.05) from the proteoglycan treatment with sodium hydrosulphate binders. However, both are real (P<0.05) higher than the PgKA and PgKS treatment.

The digestibility of dry matter shows the portion of nutrients other than digestible water stated that there are three categories of feed quality based on the level of digestibility, namely low quality with a digestibility value in the range of 50-60 percent, medium quality with a digestibility value in the range of 60-70 percent, and high quality with a digestibility value above 70 percent [11,12,13]. From the results of this study, it shows that proteoglycans of the type of potassium sulphate, have high quality in terms of digestibility. This is understandable because potassium (K) ions play a very important role in helping absorption and maintaining osmotic pressure. K ions also maintain the permeability of cell membranes so that traffic goes in and out of food juice [14,15].

When viewed from its characteristics as a constituent of amino acids, products that tend to be alkaline are more profitable. This is because many types of essential amino acids are alkaline, such as lysine. Fermented products are generally acidic. By giving products that tend to be alkaline, it will quickly help neutralize intestinal taste, which is more beneficial [16,17].

The use of bioprocessed *proteoglycan* products (from Chemical extraction) PgKP has a higher protein digestibility value than PgBP. The difference in digestibility value is caused by a difference in the amount of dissolved nutrients when extracting more chitosan, so that when the nutrient is stratified by potassium sulphate ions, there are more.

Proteoglycan products of PgKS bioprocess products have the lowest digestibility value. According to [18] the difference in digestibility value is due to differences in the properties of the processed feed, including its suitability to be hydrolysed by broiler digestive enzymes. The use of sulphate ion binders from sodium hydrosulphate tends to have a lower protein digestibility than ammonium sulphate and potassium sulphate. This is because the pH of PgKS proteoglycan products is lower (tends to be acidic) than the other two types of binders. The results of the first year's

study showed that the final pH of the proteoglycan product from the naturation of the $NaHSO_4$ binder was 6, while the ammonium sulphate naturation result had a pH of 7 and the result of naturation by potassium pH was 10 [19,20]. According to [21] amino acids formed/acidic are aspartic and glutamate (pH 4) while the amino acids that are alkaline are lysin (pH 10.5), arginine (pH 12.5), cystine (pH 8.4), and tyrosine (pH 10.5). The alkaline amino acids are more essential than aspartic and glutamate which are non-essential because they can be formed in the poultry body [22,23].

3.2. Treatment of Hematologic Value of Broiler Chicken Blood

The hematologic value of broiler chicken blood obtained as an influence of proteoglycan products can be seen in Table 2.

Table 2 Average Value of Hematologic Blood Value of Broiler Chickens in Each Treatment

Treatment	Erythrocyte	Leukocyte	Haematocrits
	(x 106 grains/mm 3)	(x 10 3 grains/mm3)	..... (%).....
PgKA	2,37 [a]	17,56 [a]	31,25 [a]
PgKP	2,46 [a]	19,29 [a]	33,50 [a]
PgKS	2,12 [b]	15,46 [a]	27,50 [b]
PgBA	2,38 [a]	18,15 [a]	31,75 [a]
PgBP	2,44 [a]	19,68 [a]	33,00 [a]
PgBS	2,39 [a]	18,44 [a]	32,00 [a]

Note: [a, b] means different superscripts within the same column significantly ($P < 0.05$)

Based on Table 2, the average number of erythrocytes of broiler chickens in this study is within the normal range, namely for the number of erythrocytes of 2.12- 2.46 x 10^6 grains / mm^3. The normal erythrocyte count in broiler chickens is 2.0 - 3.2 x 10^6 grains/mm^3 [24]. This means that chickens do not have disturbances in the physiological system of their blood because the entire average is within the normal *range*. The number of erythrocytes per mm^3 of blood varies by species and between individuals within a single species [25]. According to [26] the number of erythrocytes is influenced by several factors including age, gender, ration quality, disease, and environmental temperature.

Proteins and minerals contained in chitosan extraction liquid waste are easily digested by broiler chickens. Increased digestion and absorption of food substances will affect the metabolic processes in the body to be smooth [27]. According to [28] states that proteins can be used to repair damaged cells and tissues in the body, metabolic processes become smooth and increase growth. With a smooth metabolism affects erythrocytes that have a longer endurance, thereby reducing the number of damaged cells and affecting the number of erythrocytes.

The number of broiler chicken leukocytes in this study was in the normal range of 15.46 – 19.68 x 10 3 grains / mm^3. According [24] the normal number of leukocytes in broiler chickens is 16 - 40 x 10^3 grains/mm^3. According to [29] white blood cells are much less numerous compared to red blood cells. Added that the number of leukocytes is far below that of erythrocytes and varies depending on the type of animal [30]. Fluctuations in the number of leukocytes in everyone are quite large in certain conditions, for example: stress, physiological activity, nutrition, age, and others. States that the increasing number of leukocytes is generally a sign of an infection or wound [26].

The results of this study illustrate that proteoglycan products contain sufficient nutrients for the protein and mineral needs of broiler chickens in each treatment so that it also affects the number of uniform leukocytes to increase the chicken's immune system [31]. According to [32] leukocytes play a role in strengthening the body's defence system from various diseases and wound infections.

The proteoglycan treatment has an unreal influence on the number of leukocytes, meaning that the product can be used as a constituent ingredient in the ration of animal protein sources in broiler chickens. Rations containing enough protein for broiler chickens can affect performance, health, and endurance. According to [33] adding protein is one of the most important elements for the growth of broiler chickens.

The high haematocrits value is due to the presence of a tendency to a high number of erythrocytes. According to [12] haematocrits values have a positive relationship with the number of erythrocytes. Added that the haematocrits value is the percentage of blood consisting of red blood cells (erythrocytes).

4. Conclusion

The conclusion obtained from the results of the study that the liquid waste of chitosan extraction from shrimp shells (both through chemical and biological processes) can be used as a source of making proteoglycans and can be used as *a feed supplement* in broiler chicken rations. The quality characteristics of proteoglycan products of different types of binders with testing of digestibility and hematologic values of broiler chickens' blood are as follows:

- Digestibility
  - Good quality (value >70%): Proteoglycans of chemical and biological processes with potassium sulphate binders.
  - Medium quality (value 60-70%): Proteoglycans of chemical and biological processes with ammonium sulphate binders, and biological process proteoglycans with sodium hydro sulphate binders.
  - Poor quality (value <60%): Proteoglycan chemical process with sodium hydro sulphate binder.

- Hematologic blood
  - The number of erythrocytes is normal (range 2.12 – 2.46 x 106 grains/mm3) for all types of products.
  - The number of blood leukocytes is normal (range 15.46 – 19.68 x 103 grains/mm3) for all types of products.
  - Normal blood haematocrits values (range 27.50 – 33.50%) for all types of products.

Suggestion

- Liquid waste from chitosan extraction can be utilized in the manufacture of proteoglycans with binders recommended using potassium hydrosulphate.
- Proteoglycan products can be used as feed supplements in the ration of broiler chickens to improve the absorption of food substances and maintain the health of livestock.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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