

TESTING OF CHEMICAL, pH AND TEST OF ANTIBIOTIC CULLED LAYING HENS MEAT.

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Abstract

The main purpose of this study was to determine the chemicals contained in the culled laying hens meat and the level of acidity (pH) and to find out whether there were Oxytetracycline and Kanamycine antibiotic residues in the culled laying hens meat so as not to interfere with the food product fermentation process (salami). The samples used were whole culled layered hens (chest and thighs). Chemical / proximate testing consisting of water content, ash content, protein content, fat content, and carbohydrates and pH test for meat. Testing of antibiotic residues is done by the Bioassay method (*Screening*). The results of laboratory testing showed that the samples of the affected culled laying hens meat were analyzed, the chemical quality of the culled laying hens meat was 69.55%, 20% protein and 8.2% fat and 1.15% carbohydrate. The results of testing on culled laying hens meat after cutting obtained pH around 5.9 - 6.0. Based on the results of testing of antibiotic residues on the culled laying hens meat, negative results were obtained for the antibiotic residues oxytetracycline (per 10 g) and kanamycine (per 10 g).

Keywords: chemical analysis, pH, culled laying hens, antibiotic residues.

1. Introduction

Chicken meat is one of the livestock commodities that have nutritional value parallel to the nutritional value of other meat. Culled laying hens are an animal protein source that has not been widely used. Although it is known that low-quality culled laying hens, it is still possible to provide donations. High protein content and low fat, deserves to be directed as food ingredients. Respected culled laying hens with low egg production of around 20 to 25% at around 96 weeks of age and are ready to be removed from the cage (Gillespie and Flanders, 2010). Culled laying hens by breeders are used as broilers for meat producers and have lower meat quality than broiler chickens, because they have specific and tough odors, and culled laying hens have the potential to become processed meat products, because having nutrient content is not much different from meat broiler chickens and have high fat content (Rasyaf, 2010). The fat content in the meat determines the quality of meat, because fat determines the taste and aroma of meat (Soeparno, 2005). However, supervision to produce high quality chicken meat, free from contamination or residues of chemicals, especially drugs and safe for consumption is very necessary.

The use of antibiotics as treatment / therapy or as feed additives can increase livestock production so that it can pursue the desired target for farmers. But on the other hand the use of antibiotics can cause several problems, if the administration of irregular antibiotics can cause residues in tissues or organs of animals. According to Bahri *et al.* (2005) almost all feed mills add antibiotics to commercial feed, so most commercial feed circulating in Indonesia contains antibiotics. This improper use of antibiotics is possible due to the marketing patterns of veterinary medicines in the field, where 30.80% of small-scale broiler breeders and 33.30% of small-scale layer chicken farmers who do not have veterinarians to monitor them, get drugs directly from distributors so they are worried that the use of these drugs does not follow the correct rules. In addition, farmers do not understand the time to stop a drug, resulting in the emergence of residues in livestock products (Peter *et al.*, 2002; Bahri *et al.*, 2005). Uncontrolled administration of antibiotics to livestock is very risky as a cause of the presence of antibiotic residues in the products produced, including the processed products. But on the other hand the use of antibiotics can cause a number of problems, if irregular antibiotics can cause residues in tissues or organs of animals. Then this residue can endanger the human health that consumes it so it can cause an allergic reaction which can lead to increased sensitivity, then a resistance reaction due to consuming in low concentrations for a long time. With the dangers of residual effects on health, there are provisions for the Maximum Residue Limit (BMR) in livestock products for each antibiotic based on the Indonesian National Standard (SNI, 2001). In the provisions of SNI, there is a list of types of antibiotics and their metabolites, followed by the BMR value in each animal product (meat, milk and eggs). Antibiotic residues in livestock products can be avoided if all parties pay attention and obey the rules of antibiotic use. As early as possible awareness of the dangers of antibiotic residues in food products from livestock can be the first step to reduce the risk of consumers being exposed to residues in food products from livestock consumed. Therefore the purpose of this study is to determine the chemical composition and the presence or absence of antibiotic residues in the meat of the layer laying hens besides to ensure that food products are safe for consumption, the National Standardization (BSN) sets the Maximum

Residue Limit (BMR) listed in SNI 01-6366 2000 stipulates that the maximum limit of tetracycline residues in livestock products is 0.1 mg / kg in meat and 0.05 mg / kg in eggs.

2. Research Method

Research includes; chemical / proximate testing consisting of water content, ash content, protein content, fat content, (Association of Official Analytical Chemists, 1995) and carbohydrates (Winarno, 1992) and pH test for meat (Lukman and Trioso, 2009). pH measurements are carried out using a pH meter. The purpose of the study was to determine the levels of food substances in the meat of the culled laying hens and the growth of microbes from livestock, or pollution from the environment during the cutting process. Parameters measured included chemical or proximate testing consisting of water content, protein content, fat content and carbohydrate, pH of culled laying hens and total bacterial testing and antibiotic residue testing. Proximate analysis carried out in the laboratory of Ruminant Animal Nutrition and Animal Food Chemistry and meat pH tests were carried out at the Livestock Product Processing Technology Laboratory, Padjadjaran University, Bandung, Antibiotic Residue Test was conducted at the Kesmavet Laboratory of DKI Jakarta Province using a screening test by bioassay with normal standards the diameter of the obstacle zone used is 20 ± 1 mm from the diameter of 8 mm disc paper according to the technical guidelines of the Indonesian National Standard (SNI) number 7424: 2008. The rejected laying hens used were Isa Brown strains obtained from livestock companies in Sumedang, West Java. The sample used was the whole laying hens.

Analysis of Chemical Properties.

(1). Determination of Water Content Gravimetric Method (AOAC, 1995)

- The cup used must first be weighed by inserting the cup into the oven with a temperature of 103-105 ° C for 30 minutes, then cooled in the exicator for 5-10 minutes, put in and weighed until a constant cup weight is obtained. Constant weight is the result of weighing in a row with a difference of 0.01.
- Weigh the sample as much as 3-5 grams in the cup that has been spawned.
- The cup and sample are evaporated above the water bath until it is dried and then dried in an oven with a temperature of 103-105 ° C for 2 hours; then cooled and then weighed, until a constant weight is obtained.

Calculation:

Moisture = (Weight loss) / (Initial Weight) x 100%

Weight loss = (weight of cup + sample before heated) - (weight cup + sample after heated)

The initial weight = the weight of the cup that has been constant + the weight of the sample before heated

Constant weight = The results of weighing in a row with a difference of 0.01.

(2). Determination of Protein Levels (AOAC, 1995).

Protein content in the sample was analyzed using the Kjeldahl method which is a total N concentration analysis. Weighed 0.1 grams of sample into a 100 ml Kjeldahl flask then added selenium in a ratio of 1: 1 then added 3 ml of concentrated H₂SO₄ slowly through the tube wall .The sample is reconstructed until the solution becomes clear. The destructive flask was cooled, then 50 ml of distilled water was added and 20 ml of 40% NaOH was then distilled off. The distillation results are collected in an Erlenmeyer flask containing a mixture of 10 ml 2% H₃BO₃ and 2 drops of pink pink Cresol Green Methyl Red indicator. After the volume of the reservoir was 10 ml and bluish green, the distillation was stopped and titrated with 0.1 N HCl solution to return to pink. The same treatment is tested against blank.

Protein levels are determined using the formula:

$$\% N = ((\text{Sample-Blank}) \times N \text{ HCL} \times 14,008) / (\text{mg sample}) \times 100\%$$

$$\text{Protein} = \% N \times \text{correction factor}$$

$$= \% N \times 6.25$$

N HCl = HCl normality

Description: S = sample titrant volume (ml)

B = Volume of blank sheet (ml)

W = dry sample weight (mg)

(3). Determination of Fat Levels (AOAC, 1995)

The crushed sample was weighed and then wrapped in filter paper, then placed in a Soxhlet extraction tool then extracted for 6 hours using hexane solvent. The fat collected in the flask is dried, by putting it in the oven 105 °C, then weighing it until a constant weight is obtained. Fat content is calculated by the formula:

$$\text{Fat Level (\% b k)} = 100 / (100 - \text{moisture content}) \times 100$$

(4). Carbohydrate levels (by difference) (Winarno, 1992)

Carbohydrate levels are determined by the by difference method, namely by calculating water content, ash content, protein content and fat content. The following is the equation used in calculating carbohydrate levels by the method of difference.

Carbohydrate levels (%) = 100% - (% moisture content +% ash content +% protein +% fat content)

Antibiotic Residue Test for Chicken Meat

Test Method Antibiotic Residues.

Antibiotic residues in the meat of the affected laying hens were tested using a screening test (bio-assay) with normal standard diameter of the inhibition zone used 20 ± 1 mm of diameter 8 mm disc paper according to the technical guidelines of the Indonesian National Standard (SNI) (7424: 2008). The presence of antibiotic residues can be seen by the formation of a barrier area around the disc paper. Samples of 300 grams of culled laying hens are sliced with a scalpel and put into paper discs into the slices. Petri dishes were filled with culture media (*Bacillus stearothermophilus* ATCC spores for penicillin group; *Bacillus cereus* ATCC 11778 spores for tetracycline group; *Bacillus subtilis* ATCC 6633 spores for aminoglycosides; Vegetative *Kocuria rizophila* (*Micrococcus luteus*) ATCC 9341 for macrolides) and allowed to stand until the media solidifies. Paper discs that have been put into meat are taken using sterile tweezers, and carefully placed on the surface of the compacted culture medium.

Each petri dish consists of 5 disc paper, consisting of 4 disc paper from a sample of layered chicken meat and 1 paper disc from an antibiotic solution as a standard solution. A standard solution of 75 μ l is dripped onto the disk in a perpendicular manner using a micro pipette, as a positive control of each class of antibiotics at a certain concentration in each milliliter of solution. Standard solutions of the penicillin group are represented by sodium penicillin (0.01 IU / ml). The tetracycline group is represented by oxytetracycline (1.0 μ g / ml). Gologan aminoglycosides are represented by kanamycin (1.0 μ g / ml) and the macrolide group is represented by tilosine (1.0 μ g / ml). The petri dish was incubated into an incubator with different temperatures, namely the incubator temperature tetracycline group 30°C, 36°C macrolide and aminoglycoside groups, and 55°C penicillin group for 18 to 24 hours. The reading of the results is done using the calipers; to measure the zone of inhibition that forms around the disc paper, if it contains antibiotic residues. The sample is stated to be positive containing antibiotic residues if it is formed at least 2 mm clear zone (barrier area) around the disc paper.

3. Result And Discussion

3.1. The results of the analysis of chemical culled laying hens

Meat is composed of water, protein, fat, carbohydrates, vitamins, enzymes and minerals. In general, the chemical composition of meat consists of water (65 - 80%), protein (16-22%), fat (1.5 - 13.0%), carbohydrates (0.5 - 1.5%) and solutes not protein (2.3%) and the rest is vitamin. Variations in meat composition can be caused by differences in growth, feed, nation, meat muscle location, storage and preservation (Lawrie, 2003 and Aberle *et al.* 2001). Lukman *et al.*, (2009) reported that the chemical composition of chicken meat such as protein is 23.3%; 74.4% water; 1.2% fat and 1.1% ash. based on the results of the analysis, the chemical quality of the laying hens was obtained 69.55% water content, 20% protein, and 8.2% fat and 1.15% carbohydrate. Abubakar (2009) states that chicken carcasses are fast and easily damaged, because they contain water (65-70%), protein (19-22%), fat (10-12%), and minerals (1 - 2%), which is easy react, degrade, promote enzyme activity and is a good medium for microbial growth and development.

In contrast to broiler chicken meat contains high nutrition, such as protein 18.2 g / 100g broiler chicken meat, while the fat ranges from 25.0 g. (MOH, 1996). The quality of the carcass of the culled laying hens is less attractive to consumers, considering that the fat content is relatively high and the meat has a harder / tough nature. But the meat has a potential laying hens to be processed meat products, because they have nutrient content not much different from broiler chicken meat even though it has high fat content (Rasyaf, 2010). The fat content in the meat determines the quality of meat, because fat determines the taste and aroma of meat (Soeparno, 2005). Therefore, the reject layer laying meat is an alternative for processed meat products, which are made with sausages, nuggets, shredded meat, etc. by increasing the quality of taste, storability, nutrition and physicality of raw meat.

3.2. pH Testing Results

With the cessation of blood circulation after the cattle are cut, it will cause the cessation of blood function as an oxygen carrier, so that respiration stops and the process of anaerobic glycolysis takes place. Based on the results of testing the reject chicken meat after cutting, pH was obtained around 5.9 - 6.0. According to Lukman *et al.* (2009), the pH value in chicken muscles at the time of cutting is about 7.0 and decreases during anaerobic glycolysis (postmortem glycolysis) to 5.5-5.9. The range of pH value of chicken meat after rigor mortis is 5.5 to 6.4. The final pH value of chicken meat is reached about 3 hours after cutting and a good final pH value in chicken meat between 5.5-5.9. The final pH value of the meat will determine other characteristics of meat

quality, such as muscle structure, water binding capacity, microorganism growth, protein and enzyme denaturation, meat tenderness, and meat emulsification capacity. The results of the study by Duna *et al.* (1993) that the average initial pH of broiler chest muscle was 7.09 and then decreased to 5.94, that is, six hours after death. Changes in the pH of the meat after slaughtering cattle are affected by the availability of lactic acid in the muscles, the availability of lactic acid is affected by glycogen content, and the glycogen content is affected by the handling of cattle before being cut. Muscle glycogen content is very low, ie in the range of 0.5 to 1.3% of the weight of fresh meat (Soeparno, 1992), so that the decrease in meat pH occurs gradually and requires a long period of time. The occurrence of changes in pH values is thought to be due to changes in pH after the cattle die, basically determined by the glycogen content in the meat and handling before slaughtering.

Decreasing pH will affect the physical properties of meat. The rapid rate of decrease in muscle pH will result in low water binding capacity, due to the increased contraction of the formed actiocytes, thereby squeezing the liquid out of the meat. High temperatures can also accelerate the decline in postmortem muscle pH and decrease the water binding capacity due to increased denaturation of muscle protein and increased movement of water to the extracellular space (Lawrie, 2003). Aberle *et al.* (2001) stated that if animals move too much at the time before being cut, then the supply of muscle glycogen will decrease, because some glycogen is used for motion. In this case the livestock do a lot of motion activity so that the glycogen reserves in the meat muscle are small. Due to the low production of lactic acid, the pH of the meat will slowly rise. The pH of fresh meat depends on the muscle glycogen content when cutting. This is supported by the opinion of Buckle *et al.* (2010) that which determines the final pH of the meat is the size of the glycogen content in the muscle when cutting.

3.3. Results of Testing of Antibiotic Residues in Culled Laying Hens Meat.

Antibiotics are substances or substances produced by certain microorganisms that can be used for treatment of infections caused by bacteria. These compounds are able to stop the growth process of bacteria and can even kill bacteria which are commonly known as bacteriostatic and bactericidal effects (Bezoen *et al.* 2000). Detection of antibiotic residues in the study aimed to determine whether there were Oxytetrasyklin and Kanamycine antibiotic residues in the meat of the rejected laying hens. The samples used were whole layered hens (chest and thighs). Sample testing was carried out using Bioassay method (Screening) at Kesmavet Laboratory in DKI Jakarta. The results of laboratory testing showed that the samples received were negative results on antibiotic residues, both Oxytetrasyklin (per 10 g) and Kanamycine (per 10 g). Based on SNI 01-6366-2000 BMR (Maximum Limits of Residue) Oxytetrasyklin in livestock products is 0.1 (mg / kg) in meat and the maximum Kanamycine limit is 0.05 (mg / kg) in meat. Based on the above test results indicate that the sample to be used in research is free from antibiotic residues or safe for consumption

Inappropriate use of antibiotics, such as prolonged use or excessive use can result in accumulation of antibiotics in animal tissues or organs. The accumulation (residues) of antibiotics in the animal's body will have an impact on the health of humans who consume food from animals, such as allergies, poisoning and resistance to these antibiotics. Observations in the field showed that the use of antibiotics, especially in broiler and laying farms, tends to be excessive and inappropriate, without regard to the correct usage rules (Bahri *et al.*, 2005). Adam (2002) antibiotic residues caused by the use of antibiotics to control or treat infectious diseases do not pay attention to drug downtime, use of antibiotics that exceed the recommended dose, such as the use of antibiotics as feed additives in animal feed. Furthermore, it is said that improper and improper use of antibiotics from the selection of antibiotics, dosage and duration of use for the treatment of livestock or as promoters and additives in animal feed will cause residues in livestock products such as meat, milk and eggs.

In the livestock industry, the administration of antibiotics in addition to prevention and treatment of diseases, is also used as feed additives to stimulate growth (growth promoter), increase production, and increase the efficiency of feed use (Bahri *et al.* 2005). The use of antibiotics can be bad, because it can cause antibiotic residues in chicken meat. The danger of antibiotic residues can be a serious problem, especially if it enters the consumer's body, besides giving a carcinogenic effect the long-term consequences can be failed. Regarding the prevention and control of antibiotic residues in food from animals, especially animal meat, the government is expected to increase supervision of the quality and safety of food from animals from livestock to consumers. The use of antibiotics continuously and for a long time through drinking or feed water in low concentrations will trigger bacterial resistance to antibiotics in livestock (Butaye *et al.* 2003).

4. Conclusion

Based on the results of the study it can be concluded that the results of the chemical quality analysis of the culled laying hens meat obtained 69.55% moisture content, 20% protein, and 8.2% fat and 1.15% carbohydrates; pH after cutting between 5.9 - 6.0. Samples of the culled laying hens obtained negative results on antibiotic residues both Oxytetrasyklin (per 10 g) and Kanamycine (per 10 g) thus indicating that the chicken meat used in subsequent studies was free of antibiotic residues and safe for consumption.

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