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Microbiological Analysis of Bali Beef with Different Aging Times

Karenina Dwi Yuliyanti[1], Kirana Dara Dinanti Adiputra[1*], I Putu Gede Didik Widiarta[1] and Andi Nurmasytha[1]

1Department of Animal Science, Faculty of Agriculture, Mulawarman University

Jl. Kuaro, Gn. Kelua, Kec. Samarinda Ulu, Kota Samarinda, Kalimantan Timur 75119 - Indonesia

*Corresponding author : kiranadaradinanti28@gmail.com

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Abstract: Microbiological analysis of meat is crucial to ensure its safety, quality, and suitability for consumption. As a nutrient-rich medium, beef supports microbial growth, which can impact its quality. This study aimed to characterize the microbiological quality of Bali beef with different aging times. This study used three types of muscles: Longissimus *dorsi, Gluteus medius*, and *Semitendinosus* from Bali beef aged ± 3 years and body weight of ± 350 kg. Samples were aged at cold temperatures for 1, 21, and 42 days. A completely randomized design with a 3x3x4 factorial was used in this study. The least Square Means test was applied if the data obtained differed significantly. The research results show that the microbial count in the meat significantly increased during aging for 21 and 42 days. Longer aging periods in this study led to an increase in the microbial count.

Keywords: *Aging; Beef; Microbiological Qualities.*

Introduction

Bali cattle, as Indonesian native cattle, have the potential to produce premium meat. Bali cattle have the advantage of being highly adaptable to the local environment and having good reproductive efficiency. Its meat is of superior quality, with good marbling and tender texture. Research by Putra (2022) shows that Bali cattle produce premium meat with tender meat quality. Further studies on Bali beef are needed to support the development of local premium meat products that are highly competitive in the international market. The need for animal protein from meat for consumption by the Indonesian population is increasing along with the increase in population. In addition, high public awareness of nutritional needs and healthy living positively impacts consuming nutritious, hygienic, safe and wholesome food (Daerobi et al., 2020). The good dietary content in beef greatly influences the development of microorganisms. Improving beef quality needs to be developed optimally, and the quality assurance of livestock products needs to be monitored until they reach consumers.

Beef is an ideal medium for the growth of microorganisms. This is because the percentage of water contained in beef is very high, around 68-75%, and beef has a pH of 5.3-6.5, which is favorable for the growth of microbes. Microbial contamination is dangerous for beef, starting when the cow is still alive, where microbes stick to the skin's surface and rumen. This high nutritional content is an ideal medium for the growth of microorganisms and enzyme activity so that meat spoils quickly. Coliform bacteria and Escherichia coli often contaminate food products, including beef. This bacteria can cause diarrhea and endanger human health. Diarrhea is a disease that is usually found in almost all regions of Indonesia (Lorens and Rahmat, 2023).

Beef with microbial content below the maximum limit is essential to ensure healthy, intact, and halal food safety. Slaughterhouses (RPH) are areas prone to contamination by pathogenic microbes. After slaughter, microorganisms present in cattle can begin to damage tissue, causing the meat to decline in quality if not handled properly rapidly. Indriyani et al. (2019) reported that 3.1% of beef samples from Banyuwangi RPH were contaminated with Salmonella spp., emphasizing strict

sanitation implementation in RPH to prevent microbial contamination. The number and type of microorganisms can determine the microbiological quality of food ingredients. This will determine the product's shelf life in terms of damage by microorganisms. Product safety is determined by the number of pathogenic organisms contained in it. Microorganism contamination of beef causes the shelf life of meat to decrease unless it is treated. One method to extend beef's shelf life is aging (Beti et al., 2020).

Aging is handling fresh meat after slaughter by storing the meat for a specific time under controlled environmental conditions in a cold room with a temperature of 0–4 °C and a relative humidity of 75–80%. There are various opinions regarding the aging time; the most common range is 14–40 days. The aging produces the desired product effectively (Dashdorj et al. 2016). Aging beef has been known to improve physical quality and extend the shelf life of meat without damaging the palatability value of beef. However, the aging of authentic Indonesian beef is still rarely done. Based on these problems, research was carried out to determine the microbiological quality of Bali beef with different muscle types and aging times.

Materials and Methods

The research was held at the Slaughterhouse, IPB Food Technology Microbiology Laboratory, and the Large Ruminant Livestock Laboratory, IPB Faculty of Animal Husbandry. The tools used are dropper pipettes, vortexes, stomachers, petri dishes, test tubes, showcases, incubators, knives, glass plates, digital scales, and cooling chambers. The ingredients used are male Bali beef with an age of ±3 years and a body weight of ±350 kg. Sampling was carried out on three types of muscles: Longissimus dorsi, Gluteus medius and Semitendinosus. These three types of muscles were selected as samples because they represent the variation of Bali beef characteristics in texture, fat content, and muscle fiber distribution. This selection allows for a more comprehensive analysis of microbiological changes in different types of muscles during aging. Other materials used are distilled water, retail solution, filter paper, plate count agar (PCA) media and tissue.

Prosedure

The procedure for handling cattle before slaughter follows cattle handling at the Slaughterhouse. Before cattle are slaughtered, an ante-mortem examination is carried out to ensure that the cattle to be slaughtered are healthy and to avoid the spread of infectious diseases (zoonosis). The livestock was slaughtered with the help of a restraining box at 22.00 WIB, and then skinning, removal of the innards, post-mortem examination, carcass splitting and sample cutting were carried out. The parts taken as samples were the Longissimus dorsi, Gluteus medius and Semitendinosus muscles. Samples were weighed at 250 g for each observation. Each treatment used four replications.

Control of microbial contamination during sampling was carried out through a series of aseptic procedures. All equipment, such as knives and tweezers, were sterilized using 70% alcohol before and after use, while the workbench was cleaned with disinfectant to prevent cross-contamination. Researchers wore sterile gloves that were changed periodically and wore appropriate laboratory clothing. Beef samples were taken using aseptic techniques, minimizing exposure to air and uncontrolled environments, then immediately placed in a sterile cool box and sealed tightly. During transportation to the laboratory, samples were stored at a controlled temperature of ±4 °C to prevent unwanted microbial growth. The sample is then inserted and hung into a showcase at 16–20 °C temperature for ±24 hours to go through the rigormortis phase. Rigormortis is a phase that occurs after livestock is slaughtered, and the muscles are still contracting, resulting in stiffness in the muscles.

The samples were then taken to the Slaughterhouse to undergo aging for 1, 21 and 42 days under controlled environmental conditions in a cold room with a temperature of 0 °C and relative humidity of 75–80%. The process of aging meat in this research is aging by wet-aging. The meat is cut, and then the pieces are placed in plastic. The plastic is vacuumed to remove air. Vacuum packaging can protect the meat from microbes and air exchange from outside to maintain meat quality. The storage process is carried out at a constant temperature to keep the meat moist and not dry. With this, the texture of the meat will be softer, also

known as tender. The next stage after aging the meat is that the sample is measured according to the observed variables. The variable observed in this research is the total plate count (TPC) test.

Total of microbes per gram = 1/(Diluting factor) x total of colonies (Jacob et al. 2018)

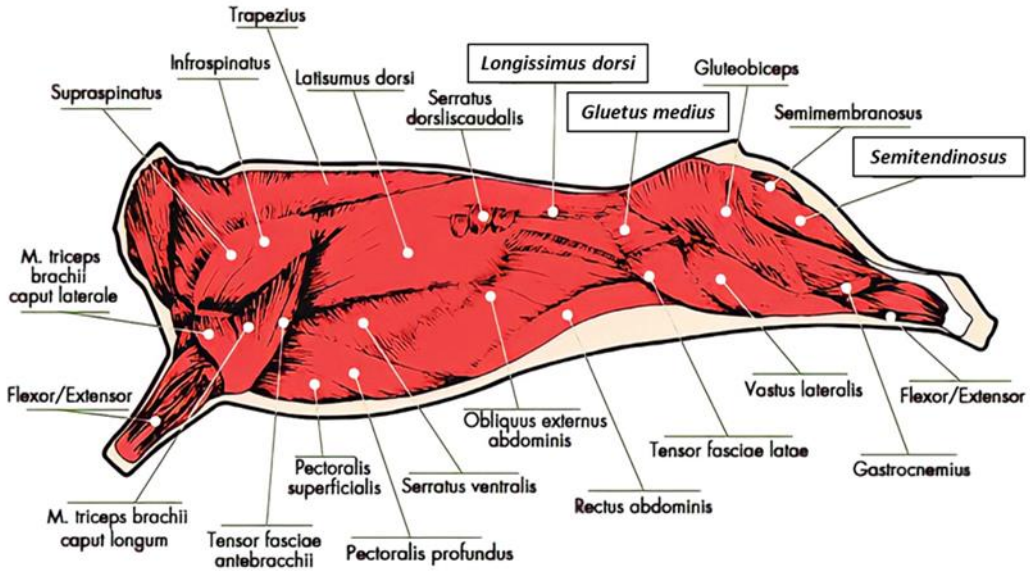

Figure 1. Muscle sections used as samples (modified from Purslow (2017) Lawrie's Meat Science. Whasington (DC): CRC Pr)

Total plate count (TPC) is a technique for calculating the number of all microbes found in meat using PCA (Plate Count Agar) media. The TPC value is a value to determine the microbiological quality of a food. The sample is ground by weighing 10 grams and then adding 90 ml of diluent solution. The sample is homogenized using a stomacher to obtain a 10-1 dilution, and further dilutions are made as needed. The sample was then shaken with a vortex until homogeneous. Samples of 1 ml were taken from each desired dilution into a petri dish and then carried out in duplicate. PCA media was poured as much as 15–20 ml for the TPC test into a petri dish. The media was homogenized by rotating the cup until it mixed with the sample. After that, the sample was allowed to freeze, and then the cup was incubated at 35–37 °C for 48 hours with the cup upside down. Next, the number of colonies was calculated.

Data Analysis

This study used a completely randomized design with a 3×3×4 factorial pattern, with the first factor being the length of aging (1, 21, 42 days) and the second factor being the type of muscle (*Longissimus dorsi, Gluteus medius* and *Semitendinosus*) with four replications. The mathematical model of this research is:

$$Yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \varepsilon ijk$$

Information:

Yijk : observations on the i-level muscle type factor, the j-level aging time factor, and the k-replication

μ : general average

αi : influence of the i-level of muscle type factor

βj : influence of the j-level of aging time factor

$(\alpha\beta)ij$: interaction effect between muscle type and length of aging

εijk : influence of experimental error

Results and Discussion

A. General Condition of The Slaughterhouse

The slaughterhouse used as a sampling site is a slaughterhouse built by the government and provides space or services for entrepreneurs who will slaughter animals. This slaughterhouse aims to provide slaughter services, making it easier to carry out antemortem and post-mortem supervision and control infectious animal diseases (zoonosis). This slaughterhouse is under the auspices of the UPTD RPH of the Food Security, Agriculture and Fisheries Service. Activities at the slaughterhouse include ante-mortem cattle examinations in holding pens carried out in the afternoon at 16.00–17.00 WIB. Cow slaughter, post-mortem examination, separation of meat and offal, aging, and deboning until the meat is ready for distribution are carried out from 20.30–05.00 WIB. Cattle slaughter is carried out at 10–20 heads/day, depending on the number of requests. The cattle slaughtered are usually Bali cattle or cattle bred in Indonesia and BX (Brahman Cross) cattle. The slaughtering process for local cattle is assisted using a restraining box, while the slaughtering of imported cattle, such as BX cattle, is carried out using a stunning process. This slaughterhouse has a level 3 Veterinary Control Number (NKV) with the number RPH-3276041-016. Slaughterhouses require an NKV certificate because it is valid written evidence of the fulfillment of hygiene and sanitation requirements as a form of guarantee of the safety of food products of animal origin.

B. Total Plate Count Test

Aging is the most common method used to improve beef quality. Aging in slaughterhouses is generally carried out at room temperature. In contrast, in slaughterhouses with more complete facilities, aging is carried out by storing beef in a closed room at a temperature of 0–4 °C for 48 hours. Aging in a closed room is carried out after the beef has passed the rigor mortis phase. Beef after passing the rigor period will be tender, but before passing the rigor period, there is a decrease in beef tenderness (Zahro et al. 2021). Wet-aging, which is aging using vacuum packaging on beef, can produce stable and optimal beef while maintaining the hygiene value of the beef. The biochemical process of wet-aging in beef has much influence on changes in the microbiological quality of the beef. Beef microbiological quality testing is carried out using the TPC test.

TPC parameters are very important to note because they are closely related to the health and safety of the food products being tested. The presence of bacterial activity in meat will reduce the quality of the meat which is indicated by changes in the meat (Fikri et al., 2017). Total microbes is a way of calculating the number of all microbes found in meat using PCA (Plate Count Agar) media (Samudra et al. 2016). Research by Aramadani et al. (2019) showed that the longer the aging, the number of bacteria increased. Aging beef for 2 days at cold temperatures produced $1.7x10^6$ microbes, while aging for 6 days produced $1.5\ x10^7$ microbes. The results of the study by Djatiwidodo et al. (2023) showed that storage at low temperatures with vacuum packaging reduced the number of microbial colonies and maintained the organoleptic quality of beef.

Another aspect that causes microbial contamination in beef is the water source used during the slaughtering process at the RPH. According to Arieta et al. (2014) that animal-based food products are very susceptible to contamination from the slaughtering area such as water, dust, soil, and air. Fikri et al. (2017) stated that to avoid cross-contamination through the floor, skinning should be done by hanging the carcass first and sterilizing the supporting facilities that will be used. Tolistiawaty et al. (2015) also stated that post-slaughter is a critical point for microbial contamination, namely during skinning, removing offal and during packaging. Cross-contamination from employee personnel and water moves to the carcass and vice versa (Ollong et al. 2020). Personal hygiene is part of the

occurrence of cross-contamination which affects the quality and safety of the products produced. Data regarding the average number of microbes in Bali beef is presented in Table 8.

Table 8 Average TPC test for three muscles with different aging times (CFU g-1)

Aging times (days)	Muscle type			Average
	Longissimus dorsi	*Gluteus medius*	*Semitendinosus*	
1	$1{,}40x10^{7}\pm1{,}15^{cd}$	$1{,}78x10^{6}\pm0{,}05^{fg}$	$9{,}30x10^{5}\pm0{,}01^{g}$	$5{,}57x10^{6}\pm7{,}31$
21	$2{,}12x10^{7}\pm1{,}50^{c}$	$1{,}10x10^{7}\pm0{,}00^{de}$	$3{,}38x10^{6}\pm0{,}17^{efg}$	$1{,}19x10^{7}\pm8{,}97$
42	$9{,}68x10^{6}\pm0{,}22^{def}$	$4{,}58x10^{7}\pm9{,}18^{b}$	$1{,}35x10^{8}\pm5{,}77^{a}$	$6{,}35x10^{7}\pm64{,}51$
Average	$1{,}50 \times 10^{7}\pm5{,}85$	$1{,}95x10^{7}\pm23{,}19$	$4{,}64x10^{7}\pm76{,}71$	

Different superscript letters in the same column indicate a significant difference (P<0.05)

The results showed that the length of aging and different muscle types significantly affected the number of beef microbes (P<0.05). The number of beef microbes increased significantly when aging for 21 and 42 days. Aging over a longer period in this study increased the number of microbes. This is the opinion of Dewi (2012), who states that the longer aging occurs, the more the number of microbes will increase. According to this research, the number of microbes in beef from the Longissimus dorsi muscle increased during aging from 0 days of $3.3x10^{6}$ CFU g^{-1}, 28 days of $9.3x10^{6}$ CFU g^{-1} and 56 days of $2.0x10^{7}$ CFU g^{-1}. Factors that can potentially cause microbial contamination of beef include contamination from the hands of the slaughterer and contamination from the floor of the slaughterhouse (Rananda et al. 2016). In addition, cutting the carcass into small pieces can expand the surface of the beef, so the possibility of contact with microorganisms is greater (Sukmawati and Hardianti 2018). Another supporting factor that causes microbial contamination of beef is unhygienic equipment, distribution and storage (Gaznur et al. 2017). Figure 2 shows the TPC results for three types of muscles.

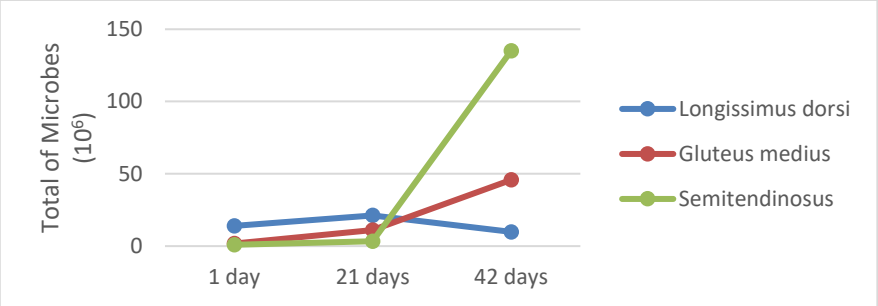

Figure 2 Graph of TPC test at different aging times

The interaction between aging treatment and muscle type had a significant effect (P<0.05) on the number of meat microbes. The number of microbes in the Longissimus dorsi and Gluteus medius muscle was more significant than in the Semitendinosus muscle at the beginning of aging. This is thought to be because there has been contamination by microbes in the muscles while in the slaughterhouse. The opinion of Gaznur et al. (2017) states that the factors causing microorganism contamination in beef can be caused by the sanitation and hygiene of slaughterhouses, which do not ensure cleanliness. Also, beef can be contaminated with microbes during sampling and cutting in the laboratory. The opinion of Kuntoro et al. (2013) states that everything that comes into direct contact with meat, such as tables, utensils, knives, cutting boards, and the environment, can be a source of contamination. Ahmad et al. (2013) also stated that contamination can occur if the cutting and transportation equipment is unhygienic.

Based on the graph above, it can be seen that TPC microbial contamination in fresh meat is above the maximum threshold according to SNI 7388:2009. According to SNI7388:2009, the maximum limit for microbiological contamination in beef against TPC contamination is $1x10^6$ CFU g-1. This proves that fresh beef has been contaminated with microbes starting from the slaughtering process until the carcass or meat is produced at the slaughterhouse. Observation results show that meat contamination factors in the slaughterhouse are caused by several things, including: (1) officers smoking, eating and drinking in the slaughterhouse, (2) foot dipping at the entrance to the slaughterhouse not filled with disinfectant, (3) worker traffic from dirty areas to a clean area or otherwise not well organized so that cross contamination is very likely to occur, (4) there is no hand washing place available, and workers do not use boots, gloves and masks, (5) the condition of the main room of the slaughterhouse and the equipment used is not in good condition. Sterile conditions and not disinfected after use. One of the initial indicators of contamination in fresh beef can be seen from the number of TPC because these bacteria occur naturally in fresh beef and can cause disease if their presence is above the permitted threshold. Beef contamination that exceeds normal standard limits will cause a decrease in the quality of the meat produced (Sukmawati and Hardianti 2018). Handling beef must pay attention to good hygiene and sanitation standards.

Conclusion

The research results showed that aging for a longer time increased the number of microbes. All beef samples taken from slaughterhouses had above-average values for microbial contamination. According to SNI, the maximum limit for microbiological contamination in beef against TPC contamination is 1×10^6 CFU g^{-1}. This contamination is influenced by cross-contamination of beef, equipment, and the aging process. Beef contamination that exceeds normal limits will lead to a decline in meat quality and pose potential health risks to consumers. Therefore, the meat industry must implement and strictly enforce comprehensive hygiene and sanitation standards at all stages of meat processing, from slaughtering to distribution. This includes regular disinfection of equipment, proper handling and storage, strict temperature control, and worker hygiene training. Additionally, continuous monitoring and microbiological testing should be conducted to ensure compliance with safety standards.

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