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Estimation of Marbling Score by Ultrasonography and Visual Methods of Male Bali Beef Based on Muscle Location and Storage Time

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A B S T R A C T

Marbling refers to white fat granules within muscle fibres that enhance meat tenderness and flavour. This study aimed to determine the marbling score of male Bali beef using two methods: ultrasonography and visual assessment based on muscle location and storage duration. Five intensively raised male Bali cattle (average weight 227 kg) were examined. Visual assessment used 0.5 kg samples from sirloin, rib, and round muscles, analysed with ImageJ and Minitab 17. Results showed that both muscle location and storage duration significantly affected ($P < 0.01$) the marbling score. The ultrasonography method yielded marbling scores of 1.06–1.44, while the visual method produced 1.10–1.50. The ultrasonography method proved reliable for predicting marbling without slaughter, with higher marbling observed in passive muscles (sirloin) than in active muscles (rib and round).

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

1. INTRODUCTION

1.1. Research Background

Indonesia is known for its abundant diversity of livestock genetic resources, one of which is the Bali cattle. Bali cattle are one of Indonesia's native germplasms, originating from the domestication of banteng (Bos javanicus) and having genetically adapted to tropical environments. This cow exhibits distinctive characteristics, including a reddish body colour, a black dorsal line, and dense muscle development [1]. As one of the national genetic assets, Bali cattle possess several advantages over other cattle breeds, including high adaptability to tropical environmental conditions, disease resistance, reproductive efficiency, and the ability to utilise low-quality feed [2].

Red meat is an important part of the human daily diet, because it contains micronutrients (iron, zinc, selenium, potassium) and various B vitamins including niacin, riboflavin, thiamine, and vitamin B12 [3]. This nutritional content makes Bali beef a potential source of animal protein to meet the nutritional needs of the Indonesian people. One important parameter determining meat quality is marbling, which refers to the distribution of white intramuscular fat within muscle fibres [4]. The marbling score can be used as a basis for assessing meat quality based on the distribution and number of intramuscular fat granules [5].

Meat quality is one of the main factors that determines the level of consumer acceptance of beef products. Quality meat typically has a medium to high marbling score, a tender texture, a fresh red colour, a distinctive aroma, and a delicious taste [6]. Beef circulating in the Indonesian market still exhibits significant

variations in quality, as most of it still originates from traditional slaughterhouses that have not implemented optimal quality control standards [7]. Variations in meat quality can be caused by various factors, including differences in breed, age at slaughter, feed, physiological condition of livestock, storage methods, and post-slaughter handling methods [8].

Conventional marbling assessment is typically performed using a visual method with a score comparison sheet; however, this approach is subjective due to its high dependence on the observer's perception [9]. Along with technological developments, the ultrasound method has begun to be applied to assess marbling in both live and post-mortem livestock [10]. This approach enables more objective and accurate measurements, particularly when the resulting images are analysed using digital-based software such as ImageJ, which is widely utilised in biomedical research and meat texture analysis [11]. Ultrasonographic image analysis using ImageJ can be used to estimate marbling scores and intramuscular fat content in Bali and Sumba Ongole cattle more consistently and quantitatively [12]. This study was conducted with the aim of determining the marbling score of male Bali beef using both ultrasonography and visual methods, based on muscle location and storage duration.

1.2. Literature Review

One of the key parameters that determines meat quality is marbling, specifically the distribution of white intramuscular fat within muscle fibres. Marbling is a primary indicator of beef quality because it directly influences the tenderness, taste, aroma, and juiciness of the meat when cooked [13]. Intramuscular fat also plays a crucial role in determining the cholesterol profile of meat, as it is the primary site for cholesterol accumulation, which can change due to the cooking and storage processes [14].

Several previous studies have demonstrated a positive relationship between the level of marbling and the taste and consumer acceptance of beef [15, 16, 17, 18, 19]. Meat with good marbling generally has a more savory flavour and a softer texture due to the distribution of fine fat within the muscle tissue. Marbling assessment is an essential aspect in evaluating beef quality, including Bali cattle, which are known for their compact muscles and relatively low fat content.

Meat quality is influenced by genetic factors, assessment methods, and the conditions under which animals are raised and slaughtered. Factors influencing livestock before slaughter can include genetics, gender, age, feed, and stress levels [20]. The physiological and psychological condition of livestock before slaughter is closely related to the final quality of the meat, including colour, pH, and tenderness [21].

Muscle location and storage time are also important factors that influence the marbling score of meat **[2].** Each muscle has a distinct fibre structure and fat composition, which in turn affects the characteristics of marbling, texture, and tenderness of the meat. More active muscles generally have lower levels of intramuscular fat than muscles that are rarely used. Storage time can impact meat quality through biochemical processes, including fat oxidation, protein denaturation, and pH changes, which can ultimately compromise the sensory quality and nutritional value of meat **[22].**

1.3. Research Objective

Most previous studies have examined the effects of genetic factors, feed, or maintenance systems on marbling scores and meat colour using conventional methods. The study of muscle variation and meat storage duration on marbling scores using ultrasonography during storage is a crucial research objective to be carried out in order to obtain accurate data on Bali beef marbling scores.

2. MATERIALS AND METHODS

Sampling of Bali cattle research was conducted at Pemenang Slaughterhouse, North Lombok Regency, West Nusa Tenggara, for ante-mortem examination using ultrasonography and meat sampling for visual observation of meat marbling. The study used 5 male Bali cattle with an average body weight of 227 kg which were intensively raised. Meat samples were taken from three muscle locations — rib, sirloin, and round — each amounting to 0.5 kg, which were then divided into three groups based on storage time (0, 1, and 2 weeks) at a temperature of -18°C, resulting in a total of 45 samples.

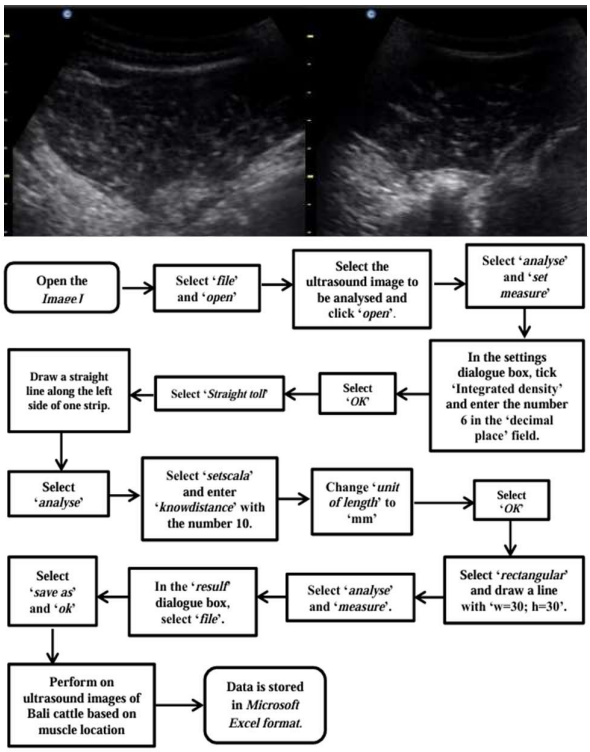

Figure 1. Steps for estimating marbling scores using ImageJ and saving measurement data in Microsoft Excel from ultrasound images [12].

Ultrasonography image acquisition was performed using a 5 MHz frequency transducer with a depth of 180 mm, which produced a sonogram image for marbling analysis. The marbling score estimation was analysed using ImageJ software with region of interest (ROI) areas measuring 15 × 15 mm, 30 × 30 mm, and 45 × 30 mm, with the estimation steps as shown in **Figure 1.**

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The image results, compared with the Australian Meat (AUS-MEAT) marbling reference standard, which has been converted to a black and white image using Corel PhotoPaint X7, are shown in **Figure 2**.

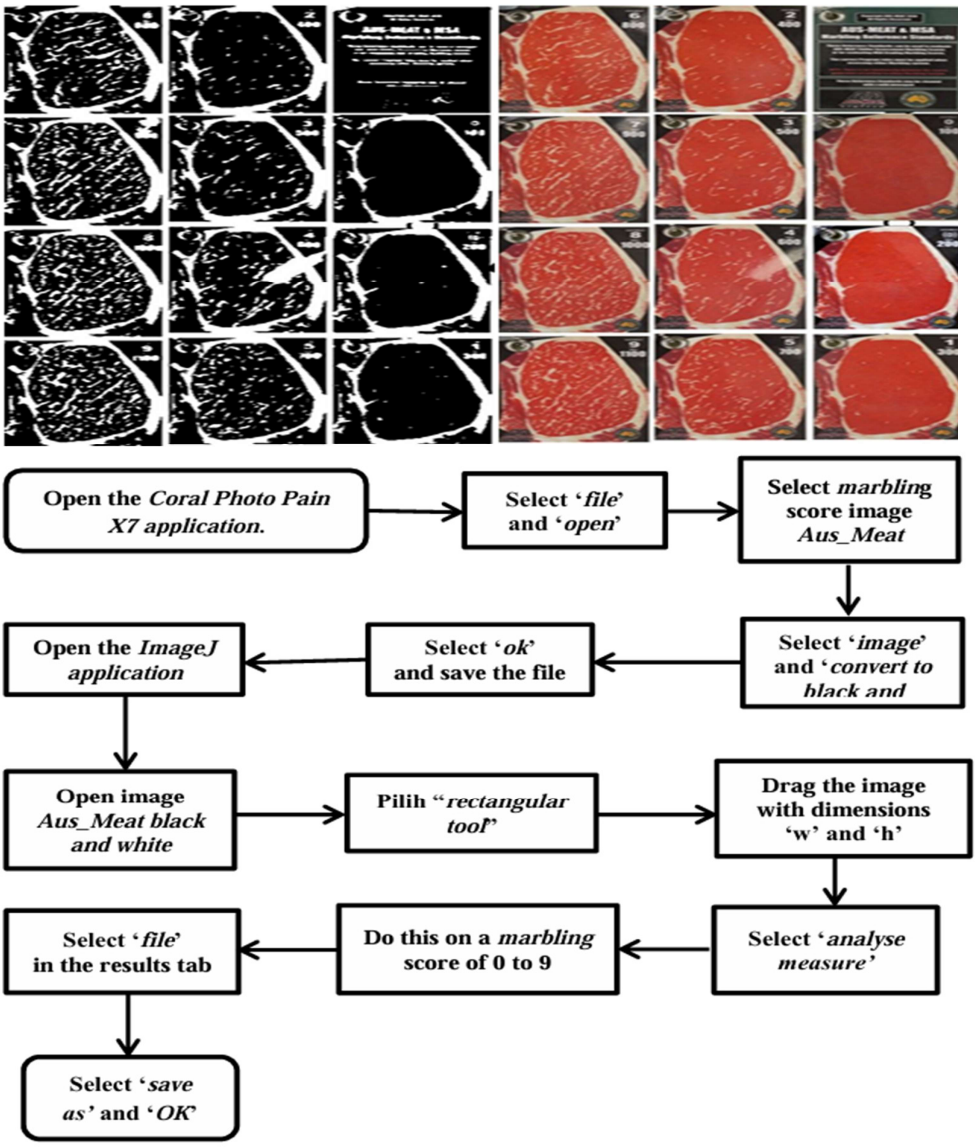

Figure 2. Steps for determining the reference for comparing marbling scores using the Corel Photo Paint X7 program [12].

The integrated density value from the image results and reference scores was analysed using the Minitab 17 program to determine the regression model used as the basis for estimating the marbling score in male Bali beef, with the steps outlined in the flow diagram (**Figure 3**).

The mathematical model used is: (y = a + bx) [23]
Explanation:
y = *marbling* score;
x = *integrated density*;
a = intersection point of the line with the y;
b = slope of the line

The data from the marbling score research using the ultrasonography method and visual were analyzed using Analysis of Variance based on a one-way Completely Randomized Design, while the marbling score of Bali beef based on storage time (factor A) and muscle type (factor B) was analyzed using Analysis of Variance based on a 3x3 Completely Randomized Design factorial pattern and continued with the Duncan Multiple Range Test.

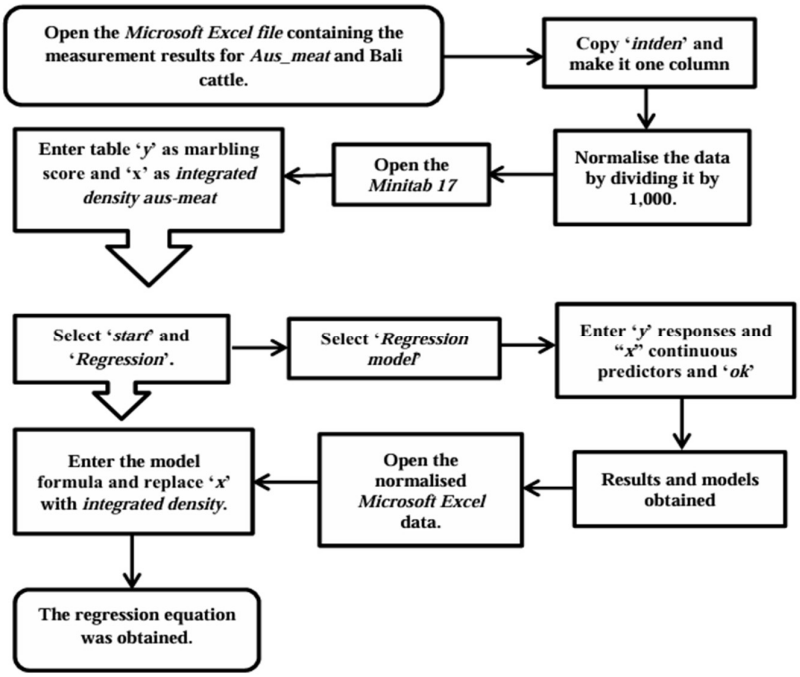

Figure 3. Steps for determining the marbling score regression model using the Minitab17 and Microsoft Excel programs [12].

3. RESULT AND DISCUSSION

3.1. Marbling score using ultrasonography and visual methods

Based on the results of a regression analysis of marbling scores for male Bali cattle at different muscle locations using ultrasonography with the Minitab 17 program, the regression model equation obtained was y = 0.856 + 0.147x, with an R^2 value of 0.713. while the visually method using Minitab 17 yielded the regression model equation: y = 0.961 + 0.316x with an R^2 value of 64.5%. These regression equations were obtained by entering y (marbling score) as the response and x (integrated density) as the continuous predictor. Based on the above regression equation, it can be used as an accurate analysis tool to obtain marbling scores. It is reported that a regression model with an R^2 value approaching 100% can be used to calculate accurate meat marbling scores [24].

The marbling score of Bali beef, as determined by ultrasonography and visual methods based on regression equations for different muscle locations, is presented in Table 1.

Table 1. Marbling Score of Male Bali Beef Using Ultrasonography and Visual Methods

Parameter	Metode	Lokasi otot			Sig.
		Rib	Sirloin	Round	
Marbling	USG	1.06 ± 0.05^{a}	1.44 ± 0.05^{c}	1.30 ± 0.00^{b}	0.000
	visual	1.10 ± 0.06^{a}	1.50 ± 0.03^{c}	1.30 ± 0.02^{b}	0.000

Description: Superscripts ^{abc} on the same line indicate a highly significant difference (P<0.01), Sig=significance; USG= Ultrasonography

The results of the analysis of variance showed that the marbling scores obtained using the ultrasonography method and the visual method were significantly different ($P \leq 0.01$) across different muscle locations. The results of measuring the marbling score of Bali beef using both the Ultrasonography method and the visual method showed that the highest marbling score was found in sirloin meat, reaching a score of 1.44 - 1.50, followed by round meat at 1.30, while rib meat had the lowest marbling score, namely 1.06-1.10. Variations in the distribution and deposition of intramuscular fat between passive and active muscles cause the difference in marbling scores. It is said that the sirloin muscle is located at the back of the cattle's body, near the flank, and is a passive muscle compared to the rib and round muscles. As a result, it tends to store more intramuscular fat, which can lead to high marbling content [24].

The results of the study demonstrate that the marbling score can be determined even when the livestock slaughter process is not conducted using ultrasonography. The ultrasonography method can serve as an accurate alternative to visual observation

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for assessing meat quality [12]. The results of this study indicate that the marbling score of male Bali cattle raised intensively in North Lombok Regency based on muscle location (rib, sirloin and round) is lower compared to the marbling score of male Bali cattle in the transverse longissimus dorsi muscle (1.51) and longitudinal longissimus dorsi (1.53). In contrast, the marbling of Sumba Ongole cattle is transverse longissimus dorsi (2.06) and 2.28 for Longissimus Dorsi Longitudinal [12]**.** The marbling score of male river buffalo meat is (2.60), while the marbling score of female river buffalo is higher (3.67) at slaughter age of 20 to 36 months [25] and 1.84 for river buffalo meat in general [10].

3.2. 3.2. Visual method of meat marbling score based on storage time and muscle location

Based on the results of the regression analysis of the marbling score of male Bali beef based on different muscle locations and storage times using Minitab 17, the regression model equation was obtained: $y = 0.961 + 0.316x$ with an R^2 value of 64.5% (without storage); storage for 7 days: $y = 0.804 + 0.204x$ with an R^2 value of 79.2%; while storage for 14 days has a regression equation: $y = 0.7204 + 0.358x$ with an R^2 value of 11.6%. The equation obtained is then used as a reference to determine the marbling score of male Bali beef based on muscle location and storage time (0, 7, and 14 days) by entering the integrated density into the equation.

Based on the results of the regression analysis, it was found that the coefficient of determination (R^2) value differed for each storage period. Meat without storage has an R^2 value of 64.5%, which means the relationship between integrated density and marbling score is quite strong, storage for 7 days has an R^2 value that increases to 79.2%, thus indicating a closer relationship and the regression model is more accurate in predicting marbling scores, while in storage for 14 days the R^2 value decreases to 11.6%. The decrease in the R^2 value indicates that, with longer storage, the prediction of marbling scores based on integrated density becomes increasingly weak due to changes in meat quality during storage. The results of the visually marbled score measurement, based on muscle location and storage time, are presented in Table 2.

Table 2. Marbling Score of Bali Beef Based on Muscle Location and Storage Length

Muscle location	Storage time (Days)			Average ML	Sig.ML
	0	7	14		
Rib	1.09 ± 0.07	0.99 ± 0.12	0.85 ± 0.07	0.98 ± 0.02^{a}	
Sirloin	1.46 ± 0.03	1.41 ± 0.04	1.33 ± 0.04	1.40 ± 0.00^{c}	
Round	1.30 ± 0.02	1.27 ± 0.02	1.21 ± 0.02	1.26 ± 0.00^{b}	0.000
Average ST	1.28 ± 0.02^{b}	1.22 ± 0.04^{b}	1.13 ± 0.02^{a}		**(<0.001)**
Sig.ST	0.000 (<0.001)				

Description: Different superscripts abc in the same row and column indicate highly significant differences (P<0.01).Sig= signifikansi ML = Muscle location. ST = Storage time

The results of the analysis of variance showed that the marbling score, as determined by the visual method based on muscle location and storage time, was significantly different ($P \leq 0.01$). In contrast, the interaction between storage time and muscle location had no significant effect ($P \geq 0.05$). The marbling score in the sirloin muscle showed a higher value (1.40) and was significantly different ($P < 0.01$) compared to the round (1.26) and rib (0.98). This is because the sirloin muscle is an inactive muscle, resulting in higher intramuscular fat reserves within the meat fibres [26]. The marbling score of male Bali beef without storage at -180 °C was higher (1.18) and significantly different ($P < 0.01$) compared to storage for 7 days (1.22) and storage for 14 days (1.13). This is because the longer the meat is stored, the more the visual intensity of the marbling decreases, thus affecting the quality of the meat's appearance, especially fresh meat, which is visually evaluated by consumers [27]. It is further stated that differences in carcass composition can be influenced by genetic factors, age, gender, nutrition and environmental factors.

The results of visually observed marbling scores, based on meat location and storage time, were lower (0.85-1.50) compared to the marbling score of male Bali beef fed fermented pineapple skin with yeast tape in the form of herbal molasses blocks, namely 1.71-1.81 [26]. Male Bali cattle given additional feed in the form of fermented pineapple skin tape yeast concentrate can produce meat with a marbling score of 2.58-4.00 [28]. The use of fermented cocoa skin as an additional feed for fattening male Bali cattle can result in a marbling score of 3.46-3.91 [29].

Differences in feed quality and nutritional status have a strong influence on marbling formation. Bali cattle fed grain or concentrate-based feed tend to produce higher marbling than cattle that only consume field grass or green fodder [30]. Marbling variations are not only determined by biological and genetic factors, but are also influenced by image capture methods, differences in analysis tools, and data processing techniques used in the research [31; 32; 33].

4. CONCLUSION

The ultrasonography method can be used to predict the marbling score of male Bali beef without slaughtering. The marbling score of Bali beef derived from passive muscle (sirloin muscle) without storage treatment is higher than the marbling score of meat derived from active muscle (rib and round).

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