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Research article

# Meat quality, muscle fiber traits, fatty acid profiles, and sensory evaluation of Wagyu crossbred with Kamphaengsaen, Brahman, and Thai Holstein Friesian cattle

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## Abstract

This study aimed to evaluate the effects of crossbreeding Wagyu cattle with Kamphaengsaen (WKPS), Brahman (WBR), and Thai Holstein Friesian (WTHF) on growth performance, meat quality, sensory attributes, and fatty acid composition. Three groups of F1 crossbred cattle were produced by artificial insemination with Wagyu semen. The animals were reared under standardized feeding and management conditions for 11 months. Growth performance, carcass traits, pH, color, water-holding capacity (WHC), shear force, and sensory attributes were evaluated. The fatty acid profile of *longissimus dorsi* (LD) muscle and muscle fiber characteristics were assessed and histological analysis. Results shown, WTHF demonstrated higher average daily gain (ADG) and lower feed conversion ratio (FCR) compared to WKPS and WBR ($p<0.05$) while WBR exhibited the highest carcass yield percentage. WTHF showed significantly lower shear force values ($p<0.001$) and smaller muscle fiber cross-section area and fiber diameter, conversely the highest number of muscle fiber density ($p<0.001$) outperformed other groups. In terms of sensory attributes, including tenderness, juiciness, taste and overall acceptability ($p<0.001$). WTHF exhibited higher saturated fatty acid (SFA) levels ($p<0.05$), while WBR meat contained significantly higher oleic acid. All Wagyu crossbreeds exhibited higher monounsaturated fatty acid (MUFA) than saturated fatty acid (SFA) content. Crossbreeding Wagyu semen with local Thai cattle breeds significantly improved growth performance and meat quality, with distinct benefits across groups. WTHF excelled in tenderness and sensory attributes, while WBR demonstrated a favorable fatty acid profile. Wagyu crossbred with higher unsaturated fatty acid (UFA) content compared to saturated fatty acids (SFA), aligning with consumer preferences and market demands. These findings underscore the effectiveness of Wagyu crossbreeding in improving beef quality and meeting diverse consumer needs in tropical regions.

**Keywords**: Brahman, Fatty acid, Kamphaengsaen, Meat quality, Wagyu, Thai Holstein Friesian.

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# INTRODUCTION

The global demand for high-quality beef has grown substantially in recent years, driven by consumer preferences for premium cuts with superior sensory attributes. Meat quality characteristics such as marbling, tenderness, flavor, and nutritional profile play a crucial role in determining the market value of beef. Wagyu beef is renowned worldwide for its exceptional marbling, tenderness, and flavor (Park et al., 2019; Kotupan et al., 2020). However, its full blood wagyu production is often constrained by specific environmental requirements and higher production costs. Crossbreeding Wagyu with local breeds offers a practical solution to combine the breed's superior meat quality traits with the adaptability and production efficiency of indigenous cattle. This approach allows for the development of cattle suited to diverse climatic and economic conditions while maintaining desirable beef quality.

In Thailand, cattle breeds such as Kamphaengsaen, Brahman, and Thai Holstein Friesian have been integral to the beef industry due to their adaptability to the tropical climate and their potential to improve local beef production (Bunmee et al., 2018). Kamphaengsaen is known for its moderate growth and adaptability, Brahman for its heat tolerance and hardiness, and Thai Holstein Friesian for its dual-purpose use in milk and beef production. Despite their strengths, the meat quality attributes of these breeds fall short of premium standards, making crossbreeding with Wagyu a promising strategy to enhance their commercial value (Thirawong et al., 2024). Crossbreeding programs have shown potential to improve beef production in tropical regions (Wolfová et al., 2007). Breed plays a significant role in influencing beef physiology due to its impact on muscle structure (Burrow et al., 2001; Waritthitham et al., 2010). Various factors contribute to meat quality, and studies have indicated that fatty acid composition affects overall acceptability and key attributes such as tenderness, firmness, oxidative stability, flavor, and color (Milićević et al., 2014). Furthermore, it is well-recognized that muscle fiber development strongly influences meat quality. While the total number of skeletal muscle fibers is determined before birth (Picard et al., 2002). The composition of muscle fiber types changes throughout an animal's life to meet varying physiological demands (Pette et al., 1997). This muscle fiber composition impacts meat quality factors, including pH and water-holding capacity. Fatty acid content and muscle fiber development are, in turn, affected by factors such as feeding regimen, animal age, and particularly breed (Herdmann et al., 2010). This study aimed to improve the growth performance and meat quality of Kamphaengsaen (WKPS), Brahman (WBR), and Thai Holstein Friesian (WTHF) cattle through Wagyu crossbreeding. Additionally, it sought to compare the growth performance and beef quality among WKPS, WBR, and WTHF cattle, with a primary focus on differences in fatty acid composition, muscle fiber characteristics, and overall meat quality.

# MATERIALS AND METHODS

## Animal care

All procedures in the present animal experiments were in accordance with the Animal Experiment Guidelines of Kasetsart University, Bangkok, Thailand, (number OACKU 00759).

## Animals and experimental design

This experiment was conducted at Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom. The study involved three maternal hybrid breeds of Kamphaengsaen cattle (50% Charolais, 25% Brahman, and 25% Thai Native) that were artificially inseminated with Wagyu semen (Shigeshigetani, American Wagyu, Pornchai Intertrade, Thailand) to produce F1 hybrid WKPS offspring. Similarly, Brahman crossbred cattle (50% Brahman and 50% Thai Native) and Thai Holstein

Friesian dairy cattle (with at least 87.5% Holstein Friesian genetics) were also inseminated with Wagyu semen to produce F1 hybrids WBR and WTHF, respectively. Calves were weaned at 4 months and placed in the individual pens, provided with a total mixed backgrounding basal diet containing 1.5% of body weight and unlimited rice straw. All steers were tagged and vaccinated against foot-and-mouth disease (Bureau of Veterinary Biologics, Nakhon Ratchasima, Thailand) prior to the initiation of the trial, following a 2 weeks adaptation period. At 15 months old, the cattle had an average starting body weight of 328.22±38.32 kg. Seven animals from each group (WKPS, WBR, and WTHF) were individually housed and reared under uniform conditions for 11 months. They were provided with the same experimental diet, which included a basal concentrate feed amounting to 2% of their body weight (on a dry matter basis; DM), with a rice straw-to-concentrate ratio of 1:9. The cattle had unrestricted access to feed and water and were fed twice daily at 07:00 h and 16:00 h. Mineral salt blocks were continuously available to all animals. The concentrate feed was analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash content, and gross energy (GE; Kcal/kg) using AOAC (2006) methods. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured according to procedures of Van Soest et al. (1991). At the same time, total digestible nutrients (TDN) were calculated using the formula TDN (%DM) = 87.84 - (0.7 × ADF) according to Schmid et al. (1976). Non-fiber carbohydrates (NFC) were determined using the formula NFC (%DM) = 100(- %Ash -%Crude protein + %Ether extract + Crude fiber) following Sniffen et al. (1992). The metabolizable energy (ME) was estimated using NRC (2015). The components and chemical composition of the basal finishing diets are detailed in Table 1.

**Table 1** Ingredients and chemical composition of experiment diets.

| Ingredient (%) | Concentrate | Rice straw |
|---|---|---|
| Cassava chip | 38.6 | |
| Palm kernel meal | 19.1 | |
| Oil palm meal | 13.5 | |
| Soybean meal | 16.5 | |
| Molasses | 9.5 | |
| Bone meal | 0.4 | |
| Sodium bicarbonate | 0.3 | |
| Salt | 1.0 | |
| Premix[1] | 1.0 | |
| Total | 100 | |
| **Chemical composition** | | |
| Dry matter (%) | 89.52 | 90.82 |
| Organic matter (% DM basis) | 93.98 | 89.84 |
| Ash (%DM basis) | 6.02 | 10.16 |
| Crude protein (%DM basis) | 12.25 | 5.03 |
| Ether extract (%DM basis) | 3.99 | 2.32 |
| Neutral detergent fiber (%DM basis) | 21.70 | 59.13 |
| Acid detergent fiber (%DM basis) | 13.36 | 37.58 |
| Non-fibrous carbohydrates (%DM basis) | 56.04 | 23.36 |
| Total digestible nutrients, (%) | 78.54 | 61.53 |
| Metabolizable energy[2], Mcal/kg DM | 3.50 | 2.71 |
| Gross energy, kcal/kg | 4566.17 | 4059.86 |

[1)] The premix supplied per kilogram of diet: 60 mg of iron as iron sulfate, 48 mg of manganese as manganous oxide, 48 mg of zinc as zinc oxide, 12 mg of copper as copper sulfate, 0.30 mg of iodine as calcium iodate, 0.36 mg of selenium as sodium selenite, vitamin A, 25,000 IU; vitamin D, 340,000 IU; vitamin E, 1,000 IU.
[2)] The metabolizable energy (ME) was estimated using ME Mcal/kg/DM) = 1.01×DE (Mcal/kg)-0.45 and the digestible energy (DE) was estimated using DE (Mcal/kg DM = 0.04409×TDN) following to NRC (2015).

## Growth performance and carcass traits

At months 15 and 26 of the study, initial and final body weights, along with feed consumption per pen, were recorded for each intervals. These data were used to calculate average daily gain (ADG), daily dry matter intake (DMI), and feed conversion ratio FCR (DMI/ADG). Monthly records of dry matter intake were maintained to determine the average DMI. After a 12-hour fasting period, 21 cattle (seven from each treatment group) were weighed and transported to the slaughterhouse, with a transport time of approximately 150 minutes. Post-slaughter, the cattle were exsanguinated, eviscerated, and divided precisely along the midline. Each carcass was labeled, and the hot carcass weight was immediately recorded on the assembly line before the carcasses were transferred to a chilling room maintained at 4°C. The hot carcass weight excluded the head, tail, feet, and offal, and these weights were directly recorded for each animal in the respective groups. Carcass yield (%) was calculated using the formula (= hot carcass weight/live weight multiplied by 100) prior to storage at 4°C. On day 7 of chilling, longissimus dorsi (LD) muscle samples were collected from the right-side carcasses between the $12^{th}$ rib and the $2^{nd}$ lumbar vertebrae. These samples were analyzed for key meat quality parameters, including pH value, color, water-holding capacity (WHC), sensory attributes, shear force, muscle fiber characteristics, and fatty acid composition.

## pH value of LD muscle

Base on Boccard et al. (1981) at 45 minutes and 48 hours after post-mortem, the pH levels of the carcass were assessed using a calibrated pH meter (Prob model LE427, Mettler Toledo, Switzerland). The pH probe was carefully inserted into the loin area between the $12^{th}$ and $13^{th}$ ribs, ensuring it avoided contact with bone and connective tissue. Prior to measurement, the electrode was calibrated using standardized buffer solutions with pH values of 4.01 and 7.00. For each sample, three separate readings were taken at different locations, and the mean value was calculated and used for analysis.

## Color value determination

A color meter (Mini Scan EZ 4500L, Hunter Associates Laboratory, Inc., VA, USA) was utilized to assess color at 7 days (Boccard et al., 1981). Measurements were taken for CIE values, including L* (lightness), a* (redness), and b* (yellowness). The device was calibrated using black glass and white tile color standards. Prior to measurement, the samples were placed on a tray and allowed to bloom for 30 minutes. Readings were obtained from three distinct locations on each steak to ensure accuracy.

## Water holding capacity determination

Drip loss was measured following the method described by Honikel (1998), while cooking loss, including roasting and boiling losses, was assessed after the samples were calculated following the thawing loss of the sample in a refrigerator at 4°C for 20 hours, after were weighted for thawing loss. For each treatment, a portion of approximately 80g was taken from the muscle, cut perpendicular to the direction of the muscle fibers, and its initial weight (W1) was recorded. The samples were then vacuum-packed and placed in a water bath set at 75°C (Techne, Temperor, England) until the core temperature reached 72°C, which was monitored using a thermometer (EBRO, TTX 100, Germany). After cooking, the meat portions were removed and cooled under running water for 20 minutes to reach room temperature, softly blotted with paper wipes to remove moisture, and weighed. The final weight (W2) was then recorded. The meat was roasted in an ELBA oven (Type: F6ET, Model: 211-800X, Italy) at 175°C until the internal temperature reached 72°C for sensory evaluation. Cooking loss was calculated using the following formula:

$$Cooking\ loss\ (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

## Warner-Bratzler Shear force determination

The cooked samples were used to assess meat tenderness, with shear force measured according to the method outlined by Silva et al. (2017). The samples were cut into strips measuring 1.0 cm x 1.0 cm, and each strip was sheared perpendicular to the direction of the muscle fibers using a Material Testing Machine (LR5K; Lloyd Instruments, West Sussex, UK). The machine was equipped with a V-type blade and a 500 N load cell (S2M/500N, Force Transducer, HBM, Singapore) and operated at a constant speed of 60 mm/minute. Each sample was tested eight times, and the peak maximum force, recorded in Newtons (N), was used to determine tenderness.

## Sensory test

For sensory evaluation, the LD was roasted in an ELBA oven (Type: F6ET, Model: 211-800X, Italy) at 175°C until the internal temperature reached 72°C, measured using an EBRO thermometer (TTX 100, Germany). After roasting, the samples were cooled on a grill rack for 7 minutes and then cut into 1.0 cm × 1.0 cm cubes. Sensory testing was performed by eight trained panelists who rinsed their mouths with water or crackers between evaluations. The panelists assessed the samples based on six attributes: color, aroma, tenderness, juiciness, taste, and overall acceptability. A slightly modified protocol of the American Meat Science Association (AMSA, 2015), scoring system was used, with a scale of 1 to 9. A score of 1 indicated the lowest quality (e.g., extremely undesirable, bland, tough, dry, or unacceptable). In contrast, a score of 9 indicated the highest quality (e.g., extremely desirable, intense, tender, juicy, or overall acceptable).

## Muscle fiber diameter and fiber density measurement

Samples were cut into rectangular shapes measuring 10 mm × 15 mm × 5 mm, aligned parallel to the direction of the muscle fibers. The tissue samples were promptly fixed in 10% paraformaldehyde and subsequently processed for paraffin wax embedding using a standard protocol. The embedded samples were sectioned onto slides and which were then stained with hematoxylin and eosin (H&E) to visualize muscle morphology. The slides were examined under a microscope at 400x magnification, and a digital camera was used to capture the images (TOUPCAMTM, UCMOS10000KPA, TOUP Tek Photonics, Hangzhou, China). The system was calibrated using a stage micrometer (Objective micrometer, AX0001, OB-M 1/100, Olympus, Tokyo, Japan) to ensure accurate measurements of, muscle fiber cross-sectional area ($\mu m^2$), muscle fiber diameter (μm) and density (number of myofibers per 1 $mm^2$, number/$mm^2$). Six random fields were analyzed per slide, and the average values were calculated. Muscle fiber density was determined as the number of muscle fibers per 1 $mm^2$ area of the selected image. The images were processed and analyzed using image analysis software (TOUPVIEW, version 3.7).

## Fatty acid analysis

The meat fatty acid composition was analyzed following the method described by Folch et al. (1957). Lipids were extracted from 5 g of meat muscle using a mixture of 100 mL chloroform and methanol in a 2:1 volume ratio. Fatty acid methyl esters (FAMEs) were prepared as described by Morrison and Smith (1964). The lipid profile was determined using a Shimadzu GC-2030 gas chromatograph (Kyoto, Japan). Samples were separated using a capillary column (0.25 mm × 100 m × 0.25 μm, RT-2560, RESTEK, PA, USA) with helium as the carrier gas. The injector was heated to temperature of 250°C. The oven temperature program began at 50°C, which was later raised to 10°C $min^{-1}$ to 220°C, and held for 35 min, followed by a further increase at 5°C/min to 230°C, which was maintained for 20 min. A 1 μL injection volume was used, and the flame ionization detector

(FID) temperature was set to 250°C. Chromatograms were processed using LabSolution software (Shimadzu, Kyoto, Japan). Identification of fatty acids in the meat samples was performed by comparing the retention times of sample peaks with those of a FAME mixture standard (Food Industry FAME Mix, 37 Components, RESTEK, PA, USA). The results were quantified and expressed as grams per 100 grams of total fatty acids.

## Data analyses

The statistical significance of differences between means for various breeds was assessed using one-way analysis of variance (ANOVA). The analyses were conducted using SPSS version 20.0 statistical software. To identify significant differences among the groups of beef cattle, the Duncan's multiple range test was applied at a significance level of $p<0.05$. Results are presented as means along with the pooled standard error of the mean (SEM) for each treatment. Significant differences between groups are indicated by different superscript capital letters ($p<0.05$ by A, B, and C).

# RESULTS

## Growth performance and carcass traits for WKPS, WBR, and WTHF

Table 2 presents the growth performance and carcass characteristics of WKPS, WBR, and WTHF. WTHF exhibited significantly higher ADG compared to WKPS and WBR, along with a lower FCR, outperforming WKPS and WBR ($p<0.01$), there were no significant differences among the groups in live weight at slaughter, hot carcass weight, or dry matter intake. However, carcass yield was highest in WBR, followed by WKPS and WTHF ($p=0.012$).

**Table 2** Growth performance and carcass traits of WKPS, WBR, and WTHF cattle slaughter (15 to 26 months of age).

| Item | WKPS | WBR | WTHF | SEM | p-value |
|---|---|---|---|---|---|
| Initial body weight (kg) | 334.57 | 332.43 | 317.67 | 8.362 | 0.690 |
| Live weight at slaughter (kg) | 482.14 | 491.29 | 534.14 | 13.298 | 0.243 |
| Dry matter intake (DMI, kg/d) | 7.14 | 7.02 | 7.63 | 0.154 | 0.238 |
| Average daily gains (ADG, kg/d) | $0.51^{B}$ | $0.50^{B}$ | $0.71^{A}$ | 0.028 | 0.002 |
| Feed conversion ratio (FCR) | $14.15^{A}$ | $14.36^{A}$ | $10.81^{B}$ | 0.452 | 0.002 |
| Hot carcass weight (kg) | 283.79 | 299.70 | 303.11 | 8.635 | 0.645 |
| Carcass yield (%) | $58.77^{AB}$ | $60.95^{A}$ | $56.64^{B}$ | 0.631 | 0.012 |

Values are presented as mean; Wagyu × Kamphaengsaen (WKPS, n=7); Wagyu × Brahman (WBR, n=7); Wagyu × Thai Holstein Friesian (WTHF, n= 7).
$^{A,B}$ Means within the same row with no common superscript differ significantly $p<0.05$.

## pH and color of LD muscle

Figures 1 and 2 illustrate initial and ultimate pH levels and instrumental color measurements of the LD muscle in WKPS, WBR, and WTHF cattle raised under the same dietary conditions. The initial pH ($pH_{45min}$) was higher in WTHF, followed by WKPS and WBR ($p<0.05$). In contrast, the final pH ($pH_{48h}$) was significantly lower in WTHF compared to WKPS and WBR ($p < 0.001$). Regarding meat color parameters, WBR meat exhibited higher values for lightness (L*) and yellowness (b*), while WKPS displayed the highest redness (a*), with significant differences observed among the groups ($p<0.05$).

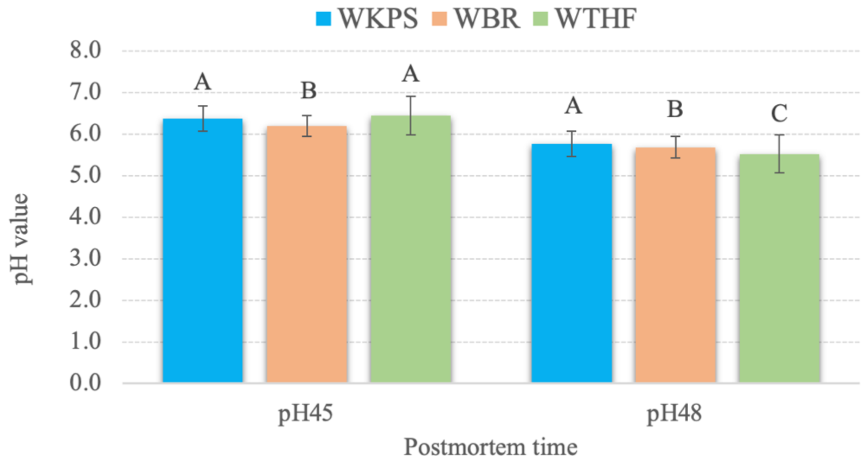


**Figure 1** pH value of postmortem in LD Muscle at 45 minutes and 48 hours. Data were mean ± standard error (SE) of Wagyu × Kamphaengsaen (WKPS, n=7); Wagyu × Brahman (WBR, n=7); Wagyu × Thai Holstein Friesian (WTHF, n= 7). Significance levels of value were shown by character A and B ($p<0.05$).

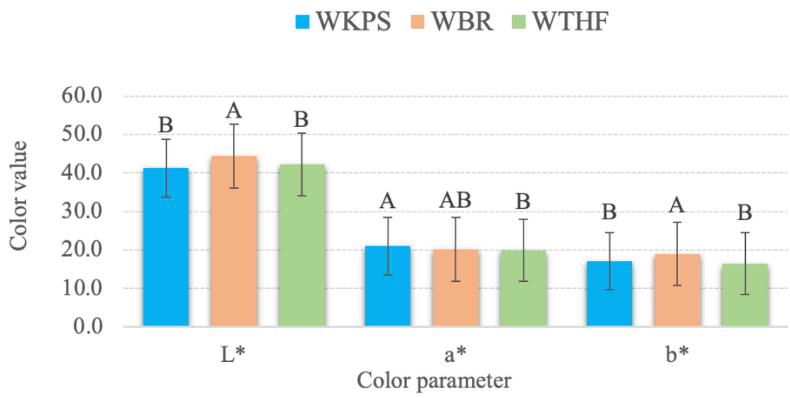


**Figure 2** Color value of postmortem in LD muscle crossbred Wagyu at 7 days. Data were mean ± standard error (SE) of Wagyu × Kamphaengsaen (WKPS, n=7); Wagyu × Brahman (WBR, n=7); Wagyu × Thai Holstein Friesian (WTHF, n= 7). Significance levels of value were shown by character A and B ($p<0.05$).

## Water holding capacity (WHC) and shear force (SF) of LD muscle

The results in Table 3 highlight distinct differences in WHC and SF values among the crossbred Wagyu groups. Drip loss and thawing loss were not significantly different across WKPS, WBR, and WTHF, indicating comparable water retention during thawing ($p>0.05$). However, WBR exhibited significantly higher roasting loss compared to WKPS and WTHF ($p<0.05$). In terms of meat tenderness, WTHF demonstrated the lowest shear force value, significantly outperforming both WKPS and WBR ($p<0.001$).

**Table 3** Water holding capacity and shear force value of WKPS, WBR, and WTHF.

| Item | WKPS | WBR | WTHF | SEM | p-value |
|---|---|---|---|---|---|
| Drip loss (%) | 1.10 | 1.40 | 1.64 | 0.148 | 0.352 |
| Thawing loss (%) | 4.74 | 4.18 | 4.86 | 0.338 | 0.693 |
| Roasting loss (%) | 22.79$^{B}$ | 27.96$^{A}$ | 22.51$^{B}$ | 0.964 | 0.024 |
| Boiling loss (%) | 26.20 | 30.14 | 26.30 | 0.775 | 0.052 |
| Shear force (N) | 45.81$^{A}$ | 43.66$^{A}$ | 32.79$^{B}$ | 1.084 | <0.001 |

Values are presented as mean; Wagyu × Kamphaengsaen (WKPS, n=7); Wagyu × Brahman (WBR, n=7); Wagyu × Thai Holstein Friesian (WTHF, n= 7).
$^{A,B}$ Means within the same row with no common superscript differ significantly p<0.05.

## Sensory test

Table 4 highlights the sensory evaluation of the LD muscle of crossbred Wagyu groups. WTHF consistently outperformed WKPS and WBR in tenderness, juiciness, taste, and acceptability with significant differences among the groups (p<0.001). In contrast, color and aroma scores remained comparable across the groups, indicating uniformity in these sensory attributes (p>0.05).

**Table 4** Sensory test attributes of WKPS, WBR, and WTHF finished under same diet.

| Item | WKPS | WBR | WTHF | SEM | p-value |
|---|---|---|---|---|---|
| Color | 7.04 | 6.86 | 7.08 | 0.060 | 0.277 |
| Aroma | 7.04 | 6.84 | 7.00 | 0.058 | 0.357 |
| Tenderness | 7.14$^{B}$ | 6.34$^{C}$ | 7.50$^{A}$ | 0.068 | <0.001 |
| Juiciness | 7.19$^{A}$ | 6.39$^{B}$ | 7.38$^{A}$ | 0.070 | <0.001 |
| Taste | 7.03$^{B}$ | 6.83$^{B}$ | 7.44$^{A}$ | 0.061 | <0.001 |
| Overall acceptability | 7.18$^{B}$ | 6.59$^{C}$ | 7.46$^{A}$ | 0.062 | <0.001 |

Values are presented as mean; Wagyu × Kamphaengsaen (WKPS, n=7); Wagyu × Brahman (WBR, n=7); Wagyu × Thai Holstein Friesian (WTHF, n= 7)
$^{A,B,C}$ Means within the same row with no common superscript differ significantly p<0.05.

## Muscle fiber characteristics of LD muscles

Figure 3 presents images of H&E strained muscle tissue, and the muscle fiber characteristics of WKPS, WBR, and WTHF cattle under the same diet are detailed in Table 5. WBR demonstrated the largest muscle fiber cross-sectional area and fiber diameter, significantly (p<0.001) exceeding those of WKPS and WTHF respectively. In contrast, WTHF showed the highest muscle fiber density, followed by WKPS, while WBR had the lowest density significance (p<0.001).

**Table 5** The cross-section of muscle fiber characteristics of WKPS, WBR, and WTHF finished under same diet.

| Item | WKPS | WBR | WTHF | SEM | p-value |
|---|---|---|---|---|---|
| Area (μm$^2$) | 1033.60$^{B}$ | 1218.98$^{A}$ | 952.29$^{C}$ | 10.763 | <0.001 |
| Diameter (μm) | 27.88$^{B}$ | 31.63$^{A}$ | 25.96$^{C}$ | 0.171 | <0.001 |
| Density (number/mm$^2$) | 569.63$^{A}$ | 370.43$^{B}$ | 648.84$^{A}$ | 13.855 | <0.001 |

Values are presented as mean; Wagyu × Kamphaengsaen (WKPS, n=7); Wagyu × Brahman (WBR, n=7); Wagyu × Thai Holstein Friesian (WTHF, n= 7).
$^{A,B,C}$ Means within the same row with no common superscript differ significantly p<0.05.

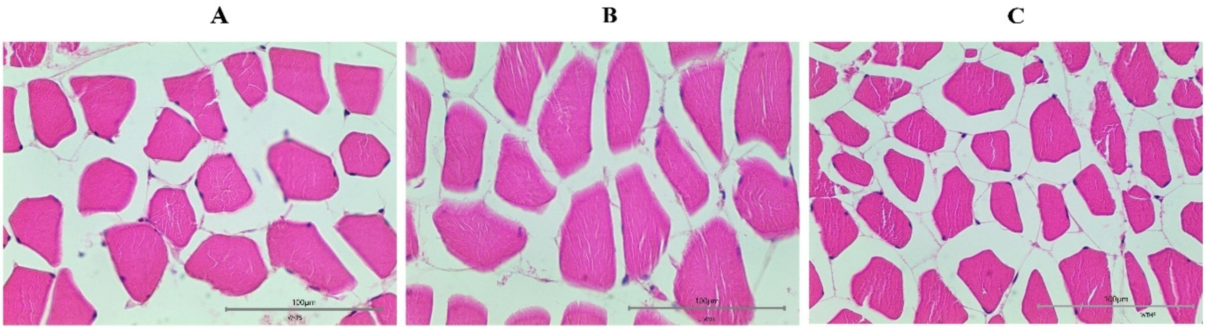

**Figure 3** The hematoxylin and eosin (H&E) staining of cross-section of muscle fiber of loin muscle fiber at 400x magnification, with a scale bar of 100 µm. The images are shown for measuring, the area of muscle fiber ($\mu m^2$), the diameter of fiber (µm), and the counted number of fiber in area of Wagyu crossbred (A) WKPS, (B) WBR, and (C) WTHF.

## Fatty acids composition and fat content

Table 6 presents the fatty acid profile of the LD muscle in WKPS, WBR, and WTHF. WTHF had the highest saturated fatty acid (SFA) was a significant difference ($p<0.05$), by the content of palmitic acid (C16:0) higher in WTHF and WKPS than WBR which was significant difference ($p<0.05$). Monounsaturated fatty acid (MUFA) were most abundant in WBR, followed by WKPS and WTHF was significant differnce ($p<0.05$). Oleic acid (C18:1n9c), a prominent of MUFA, was higher in WBR compared to WKPS and WTHF significantly diferent ($p=0.014$). Polyunsaturated fatty acids (PUFA) were detected in less quantities in WTHF compared with WKPS and WBR, However, linoleic acid (C18:2n6c) and eicosatrienoic acid (C20:3n-6) showed significant diferences ($p=0.004$ and $p=0.035$ respectively). The fat content exhibited a significant difference ($p<0.006$), with the total fat value being highest in WTHF than WKPS and WBR respectively.

# DISCUSSION

Thailand's most popular beef and dairy cattle breeds include the Kamphaengsaen, the Crossbred Brahman, and the Thai Holstein Friesian cattle. These breeds are valued for their fast growth, high fertility, adaptability to tropical regions, and ability to produce high-quality meat (Thirawong et al., 2024). However, their carcasses typically exhibit low marbling, limiting their suitability for premium beef production. On the other hand, the Japanese Black cattle, commonly known as Wagyu, are renowned globally for their exceptional marbling, tenderness, and rich flavor profile, a result of decades of selective breeding in Japan (Gotoh et al., 2018; Gotoh, 2003). To enhance the meat quality of local cattle in tropical regions, Wagyu genetics have been introduced through crossbreeding. In the present study, the WTHF crossbreed has a superior ADG and a more efficient FCR, significantly lower than WKPS and WBR. Du et al. (2017) found out that maternal nutrition plays a critical role in meat-producing animals during key gestational stages and can enhance fetal muscle and fat development (hyperplasia), leading to accumulation of fat, improved growth (hypertrophy) and production efficiency in the offspring. Previous findings indicate that crossbreeding plays a significant role in enhancing the growth performance of ruminant animals (Casas et al., 2010). However, significant differences in carcass yield were observed among the groups, with WTHF showing the lowest yield likely due to their genetic composition. Dairy breeds typically yield approximately 3% less carcass than beef breeds due to trimmed fat from abdominal deposits and visceral organs (Coyne et al., 2019). Despite these differences, there were no significant variations in live weight at slaughter, hot carcass weight, or dry matter intake among the three groups. This suggests that standardized management and feeding practices minimized differences in feed consumption and size at slaughter.

**Table 6** Fatty acid composition (g/100g) of total fatty acids and fat content of LD muscle from WKPS, WBR, and WTHF finished under the same diet.

| Item (%) | WKPS | WBR | WTHF | SEM | p-value |
|---|---|---|---|---|---|
| C14:0 | 3.87 | 3.75 | 4.18 | 0.091 | 0.168 |
| C14:1 | 1.38 | 1.50 | 1.35 | 0.100 | 0.824 |
| C15:0 | 0.68 | 0.71 | 0.62 | 0.043 | 0.668 |
| C15:1 | 0.02 | 0.03 | 0.02 | 0.003 | 0.456 |
| C16:0 | 29.26$^{A}$ | 27.58$^{B}$ | 29.91$^{A}$ | 0.326 | 0.007 |
| C16:1 | 5.42 | 5.76 | 4.80 | 0.210 | 0.192 |
| C17:0 | 0.99 | 0.94 | 1.12 | 0.033 | 0.082 |
| C17:1 | 0.65 | 0.65 | 0.76 | 0.025 | 0.108 |
| C18:0 | 11.23 | 10.55 | 12.10 | 0.417 | 0.354 |
| C18:1n9t | 0.77 | 0.72 | 0.59 | 0.038 | 0.161 |
| C18:1n9c | 42.79$^{B}$ | 45.29$^{A}$ | 42.20$^{B}$ | 0.473 | 0.014 |
| C18:2n6t | 0.18 | 0.17 | 0.18 | 0.009 | 0.816 |
| C18:2n6c | 0.94$^{A}$ | 0.92$^{A}$ | 0.67$^{B}$ | 0.038 | 0.004 |
| C20:0 | 0.07 | 0.07 | 0.08 | 0.007 | 0.752 |
| C18:3n-6 | 0.04 | 0.07 | 0.09 | 0.024 | 0.722 |
| C18:3n-3 | 0.02 | 0.01 | 0.02 | 0.005 | 0.680 |
| C20:2 | 0.06 | 0.07 | 0.10 | 0.017 | 0.581 |
| C21:0 | 0.17 | 0.17 | 0.11 | 0.014 | 0.105 |
| C22:0 | 0.04 | 0.09 | 0.05 | 0.012 | 0.153 |
| C22:1n-9 | 0.27 | 0.27 | 0.28 | 0.009 | 0.752 |
| C20:3n-6 | 0.09$^{A}$ | 0.09$^{A}$ | 0.05$^{B}$ | 0.007 | 0.035 |
| C20:5n-3 | 0.02 | 0.05 | 0.01 | 0.008 | 0.186 |
| C23:0 | 0.21 | 0.23 | 0.18 | 0.015 | 0.371 |
| C24:0 | 0.02 | 0.02 | 0.01 | 0.004 | 0.571 |
| SFA[1)] | 46.52$^{AB}$ | 44.04$^{B}$ | 48.32$^{A}$ | 0.660 | 0.027 |
| MUFA[2)] | 51.03$^{AB}$ | 53.93$^{A}$ | 49.72$^{B}$ | 0.648 | 0.022 |
| PUFA[3)] | 1.30 | 1.26 | 1.08 | 0.053 | 0.218 |
| n-6[4)] | 1.24 | 1.20 | 0.97 | 0.050 | 0.068 |
| n-3[5)] | 0.02 | 0.02 | 0.01 | 0.005 | 0.776 |
| Fat content | 8.76$^{B}$ | 9.96$^{B}$ | 12.32$^{A}$ | 0.482 | 0.006 |

Values are presented as mean; Wagyu × Kamphaengsaen (WKPS, n=7); Wagyu × Brahman (WBR, n=7); Wagyu × Thai Holstein Friesian (WTHF, n= 7); SFA[1)], saturated fatty acids; MUFA[2)], monounsaturated fatty acids; PUFA[3)], polyunsaturated fatty acids; n-6[4)], omega 6; n-3[5)], omega 3.
$^{A,B}$ Means within the same row with no common superscript differ significantly p<0.05.

In this study, the initial pH ($pH_{45min}$) was higher in WTHF, followed by WKPS and WBR, indicating that WTHF cattle exhibit slower glycolysis immediately postmortem. This may be attributed to variations in muscle energy metabolism, buffering capacity, or the intrinsic physiological characteristics of the WTHF, as noted by Choe et al. (2008) indicating that differences in fiber type composition and post-mortem muscle characteristics affect meat tenderness, with certain fiber types being more susceptible to early proteolytic degradation influenced by pH levels. The subsequent lower final pH ($pH_{48h}$) in WTHF suggests a more pronounced decline in pH, potentially due to higher glycogen reserves and increased lactic acid production (Joo et al., 2013). However, WKPS and WBR maintained higher final pH values, possibly reflecting lower glycogen availability or variations in postmortem enzymatic activities. pH is a critical factor in meat quality since it influences the biochemical environment required for protein degradation, WHC, and the development of tenderness (Choe et al., 2008). A final pH ranging from 5.4 to 5.8 is indicative of normal, tender meat (Gotoh et al., 2018). In the present study, the observed meat characteristics fall within the normal range. Meat color is a critical determinant of quality grading and significantly influences consumer preferences. In this study, WTHF exhibited the lowest redness (a*) value, potentially due to its high marbling level and fat content (Gotoh et al., 2014; Thirawong et al., 2024). While marbling enhances flavor and tenderness, it may reduce the visual appeal of the meat. However, the WKPS group exhibited high redness (a*), a trait commonly associated with premium meat quality, likely by influenced by Wagyu genetic component. Variations in color traits among the groups may result from crossbreeding effects and the blooming duration employed in the study. As noted by Wulf and Wise (1999), the lightness (L*) value of beef stabilizes after approximately 30 minutes of blooming, while redness (a*) and yellowness (b*) values require about 78 minutes to stabilize. As presented in Table 3, the thawing, boiling and drip loss value of the LD muscle were comparable among the groups. These findings align with those of Zheng et al. (2018), reported no significant differences in the LD muscle of Jinjiang (JJ), Simmental×Jinjiang (SJ), and Kamphaengsaen (KPS) steers raised on different diets. Additionally, Waritthitham et al. (2010) found that crossbreeding Charolais×Thai Native and Brahman×Thai Native cattle improved WHC and reduced shear force values compared to local breeds. Similarly, Wagyu crossbreds (50% Wagyu) demonstrated excellent growth performance, superior meat quality, and tender meat (Huffman et al., 1996; Thirawong et al., 2024). Meat tenderness is one of the most critical factors influencing consumer satisfaction and purchasing decisions. In this study, WTHF cattle demonstrated superior tenderness compared to the WBR and WKPS groups. According to Oka et al. (2002) factors affecting beef tenderness can be classified into myofibrillar effects, connective tissue characteristics, collagen content, and background toughness. Myofibrillar-related toughness is primarily influenced by myofibrillar density, muscle fiber size, postmortem tenderization, and sarcomere length, with smaller muscle fibers generally associated with more tender meat (Chriki et al., 2013). However, WTHF cattle exhibited smaller average muscle fiber sizes and lower shear force values, highlighting their superior tenderness and aligning with prior research (Chriki et al., 2013). Similarly, the sensory evaluation further validated these findings, offering valuable insights into the impact of Wagyu crossbreeding on meat quality. These results align with the SF measurements (Tables 3 and 4), providing instrumental validation of the sensory outcomes. WBR and WKPS cattle, derived from the *Bos indicus* group, exhibited tougher and firmer textures as perceived by trained panelists. In contrast, WTHF cattle from the *Bos taurus* group produced more tender meat. According to Leal-Gutiérrez et al. (2018), *Bos indicus* beef is known to shrink protein structures, leading to a chewier texture. *Bos taurus* cattle, such as WTHF, facilitate more efficient proteolysis and muscle fiber softening, improved tenderness, and overall sensory quality (Whipple et. al, 1990). Moreover, the sensory tests revealed no significant differences in the color attributes of roasted samples, consistent with the findings of Lee et al. (2021). However, eating parameters, including tenderness, flavor (aroma and taste), and juiciness, were identified as the most critical factors influencing acceptability scores among consumers (Huffman et al., 1996). Among the groups, WTHF consistently outperformed WKPS and WBR in key sensory attributes, such as tenderness, juiciness, and taste.

Fatty acid composition and fat content in Table 6. Interestingly, MUFA comprised the largest proportion of the fatty acid group, with oleic acid (C18:1n9c) being the most abundant. In contrast, WBR showed significantly higher levels of oleic acid (C18:1n9c) and linoleic acid (C18:2n-6), which are unsaturated fatty acids (UFA). The higher total UFA content in WBR, largely driven by oleic acid, highlights the critical role of stearoyl-CoA desaturase in monounsaturated fatty acid (MUFA) synthesis, potentially explaining the elevated MUFA levels in steers adipose tissues. These results are consistent with other studies on beef (Domingo et al., 2015; Papaleo et al., 2016). The higher total UFA content in steers, largely driven by oleic acid, aligns with findings that UFAs offer cardiovascular protection and reduce atherosclerosis risk (Mir et al., 2000; Nogi et al., 2011). However, Oka et al.

(2002) reported a negative correlation between MUFA levels in fat and withering height or body weight (BW). This aligns with the findings that WTHF, with greater growth potential, had higher percentages of SFA and lower percentages of MUFA compared to the other groups. In contrast, this study aligns with earlier findings which reported that Wagyu crossbreds consistently exhibit higher MUFA levels than SFA in subcutaneous, intramuscular, and loin muscle fat (Mir et al., 2000; Gotoh et al., 2014; Domingo et al., 2015). Although genetic similarities were presented in other trials, differences in feeding regimes (e.g., concentrate versus roughage diets) may have influenced fatty acid ratios. Fat accumulates from intramuscular adipocyte hyperplasia and lipid buildup in existing adipocytes, during finishing, conventional beef cattle are fed a high-grain diet to induce intramuscular adipocyte hypertrophy by increase marbling fat and fat contents (Du et al., 2017). However, the higher MUFA-to-SFA ratio in Wagyu crossbreds appears to be predominantly genetically determined rather than environmentally driven.

# CONCLUSION

This study confirms that Wagyu crossbreeding with WKPS, WBR, and WTHF improves beef quality and production efficiency in Thailand. WKPS showed moderate improvements, due to Wagyu genetics, while the WBR demonstated a favorable fatty acid profile. Moreover, WTHF excelled in growth performance, ADG, FCR, low shear force value, and higher scores sensory qualities. All Wagyu crossbreeding had a higher MUFA than SFA, making them suitable for health-conscious markets. These findings highlight the potential of targeted crossbreeding strategies to optimize beef production systems for diverse market demands and consumer preferences.

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# AUTHOR CONTRIBUTIONS

Prayad Thirawong: Conceptualization; Investigation; Data curation; Formal analysis; Writing – original draft; Writing – review and editing (lead). Lukman Abiola Oloudo: Investigation; Writing – review and editing (supporting). Patipan Hnokaew: Investigation; Writing – review and editing (supporting). Sirirat Buaphan: Investigation (supporting); Writing – review and editing (equal). Saowaluck Yammuen-Art: Conceptualization; Methodology (lead); Investigation; Data curation; Formal analysis (supporting); Writing – original draft; Writing – review and editing (lead).

# CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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