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PREFACE

In this edition, Volume 28 No 3, we proudly present articles from animal and veterinary sciences including genetic, reproduction; animal physiology; and veterinary from scientist all over the world. The articles published in this edition are:
"Fatty Acid Synthase Polymorphism of Awassi Sheep and its Impact on Fatty Acid Composition"; "Immunity and Behaviour of Lambs Born from Ewes Fed a Flushing Diet Containing EPA and DHA"; "Performances of Post-weaned Pasundan Calves Fed Extra Diets in an Extensive Grazing System"; "Polymorphism of Melanocortin-4 Receptor Gene and Its Association with Growth Traits in Bali Cattle"; "Embryo Production and Development from Superovulated Donors in Double-Muscled Cattle and Their Crosses"; "Meat Quality Characteristics of IPB-D1 Chicken and the Final Stock from Different Locations"; and "An Empirical Evaluation of Policy Options for Increasing Dairy Production in Indonesia: A System Dynamics Approach".

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, September 2023

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Fatty Acid Synthase Polymorphism of Awassi Sheep and its Impact on Fatty Acid Composition

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ABSTRAK

Merzah LH, Mohammed RG, Rhadi FA, AL-Thuwaini TM. 2023. Polimorfisme sintase asam lemak domba Awassi dan pengaruhnya pada komposisi asam lemak. JITV 28(3):152-158. DOI:http://dx.doi.org/10.14334/jitv.v28.i3.3169.

Kandungan lemak intramuskular ternak ditentukan dengan sintase asam lemak (FASN). Profil asam lemak lemak berhubungan dengan kesehatan manusia. Penelitian ini melakukan eksplorasi hubungan antara polimorfisme gen FASN pada domba Awassi dan pengaruhnya terhadap komposisi asam lemak. Penelitian ini menggunakan 100 ekor domba jantan Awassi yang berumur antara 1 sampai 2,5 tahun. DNA molekuler diisolasi dari setiap sampel darah; genotipe, reaksi pengurutan, dan alat in silico kemudian digunakan untuk mengonfirmasi varian dalam fragmen yang diamplifikasi. Terdapat dua genotipe (GG dan GA) dari gen FASN ovine (exon 2). Novel missense c.186 G>A teridentfikasi dalam genotipe GA. Genotipe GA secara signifikan (P<0,05) meningkatkan lemak intramuskular, kandungan asam lemak tak jenuh yang lebih tinggi, dan kandungan asam lemak jenuh yang lebih rendah dibandingkan dengan genotipe GG. Kumulatif dalam analisis silico menunjukkan efek merugikan dari SNP c.186 G>A pada aktivitas FASN. Genotipe GA intramuskular domba Awassi memiliki kandungan lemak jenuh relatif rendah terhadap lemak tak jenuh. Hasilnya menegaskan bahwa c.186 G>A SNP dalam variasi gen FASN ovine berpotensi berguna untuk menilai sifat-sifat karkas. Di masa mendatang, hal ini dapat mengarah pada pemilihan hewan yang lebih efisien dengan profil asam lemak yang lebih sehat, menghasilkan daging berkualitas lebih tinggi.

Kata Kunci: Domba Awassi, Gen FASN, Komposisi Asam Lemak

ABSTRACT

Merzah LH, Mohammed RG, Rhadi FA, AL-Thuwa. 2023. Fatty acid synthase polymorphism of Awassi sheep and its impact on fatty acid composition . 152-158OI:http://dx.doi.org/10.14334/jitv.v28.i3.3169.

Livestock intramuscular fat content is determined by fatty acid synthase (FASN). The fatty acid profile of the fat is of relevance to human health. Thus, this study explores the relationship between the polymorphism of the *FASN* gene in Awassi sheep and its impact on fatty acid composition. The study used 100 Awassi rams, ranging in age from 1 to 2.5 years. Molecular DNA was isolated from each blood sample; genotyping, sequencing reactions, and *in silico* tools were subsequently used to confirm the variants in amplified fragments. The results revealed two genotypes (GG and GA) of the ovine *FASN* gene (exon 2). The novel missense c.186 G>A was identified in the genotype GA. The GA genotype had significantly (P<0.05) increased intramuscular fat, higher unsaturated fatty acid content, and lower saturated fatty acid content than the GG genotype. Cumulative *in silico* analysis indicated a detrimental effect of the SNP c.186 G>A on FASN activity. The intramuscular GA genotype of Awassi sheep had a low saturated content relative to unsaturated fats. The result confirmed that the c.186 G>A SNP in ovine *FASN* gene variation is potentially helpful in assessing carcass traits, and this might lead to a more efficient selection of animals with healthier fatty acid profiles, resulting in higher-quality meat.

Key Words: Awassi Sheep, *FASN* Gene, Fatty Acids Composition

INTRODUCTION

The fatty acid synthase (FASN) gene is a potential candidate for regulating livestock meat fat composition (Kaplanová et al. 2013; Al-Thuwaini & Kareem 2022). The fatty acid composition of livestock meat impacts consumer health (Grzes et al. 2016; Dervishi et al. 2019). High consumption of saturated fatty acids (SFAs) causes high blood cholesterol levels and cardiovascular diseases (Hudson et al., 2020). The FASN gene mapped on chromosome 11 in sheep and on chromosome 19 in cattle that encodes the fatty acid synthase (FASN) enzyme (Kaplanová et al. 2013; Pećina & Ivanković 2021), which organizes de novo biosynthesis of long-chain SFAs (Shi et al. 2019; Otto et al. 2022). Moreover, most fatty acids require the FASN enzyme for animal body fat deposition (Chu et al. 2015). Recent studies revealed that genetic variants in the FASN gene are associated with animal fatty acid composition (Crespo-Piazuelo et al. 2020; Mwangi et al. 2022). A partial explanation for the variation in meat fatty acid composition between animals could be due to these polymorphisms (Esteves et

al. 2019), in which DNA variants in the FASN gene have been found to affect the animal fatty acids content in subcutaneous and intermuscular fat (Zhang et al. 2021). Genetic variation in the FASN gene has been shown to affect the fatty acid composition of livestock fat (Mwangi et al., 2022). These SNPs include SNP g.257C>T within exon 32 in the Czech sheep (Sztankoova et al. 2018), the g.17924G>A SNP in the Hanwoo cattle, the g.17924A>G SNP in Nellore cattle (de Souza et al. 2012), and SNP g.16930T>A in yaks (Chu et al. 2015). Furthermore, the significant influence of FASN g.17924A>G SNP has been confirmed with decreased SFA in commercial crossbred cattle (Kaplanová et al. 2013). Meanwhile, these variations differ between homozygous and heterozygous, which causes the variation in unsaturated fatty acids in homozygous compared with the heterozygous (Chu et al. 2015). The Awassi sheep produce milk and meat, and they are a valuable resource for many resource-poor farmers in the Middle East (Haile et al. 2019). Awassi sheep showed superior meat quality characteristics than other sheep breeds (Suliman et al. 2021). This breed has desirable carcass characteristics and meat quality, mainly due to their fat tail allowing them to have leaner carcasses and trimming fat more efficiently. Compared to more traditional sheep breeds, the fat stored in the tailed may contribute to a lower fat level in various cuts of meat (Oramari et al. 2014; Farah et al. 2019). Due to the above considerations, no research has been conducted on the relationship between the FASN gene and intramuscular fat content in Awassi sheep. Thus, this study investigated the effects of single nucleotide polymorphisms (SNPs) of the FASN gene in Awassi sheep and their influence on fat composition.

MATERIALS AND METHODS

Animal populations, determination of the fatty acid profile, and DNA isolation

The study was conducted following the international recommendations on animal care and use under Al-Qasim Green University's approval (Agri, No. 015,3,12), between October 2017 and June 2018, at the College of Agriculture / Department of Animal Production. This study included 100 Awassi sheep (aged 1-2.5 years and weighing 40 to 60 kg). A selection of animals was made based on three herds in Iraq's middle Euphrates region. Each flock studied included 10-12 rams mated randomly with about 20-25 ewes per ram. Animals were raised on seasonal grass-fed with concentrated foods (2.5% of their body weight, 59% barely, 40% bran, and 1% salt) and freshwater until slaughter. All samples were slaughtered at abattoirs of Babylon. From each animal, the longissimus dorsi (LD) muscle samples (~100 g) were taken at 45 min postmortem between the 12 and 13th ribs, collected, and processed to determine the fatty acid profile. Fatty acid content was analyzed by the high-performance liquid chromatography (HPLC) method, according to Salimon et al. (2017). Then, the values of saturated fatty acids (SFAs) (Caprylic C8:0, Capric C10:0, Lauric C12:0, Myristic C14:0, Palmitic C16:0, Stearic C18:0, and Arachidic acid C20:0), monounsaturated fatty acids (MUFAs) (Ricinoleic C18:1cis, Ricinelaidic C18:1trans, Oleic C18:1n-9, Petroselinic C18:1n-9, Elaidic C18:1n-9, and Vaccenic C18:1n-7), and polyunsaturated fatty acids (PUFAs) (α-linoleic C18:2n6, α-linolenic C18:3n-3, β-linolenic C18:3n-3) were calculated. Blood was collected (5 ml) from the jugular vein to extract genomic DNA by the high salt method of Al-Shuhaib (2017).

PCR amplification and SNP genotyping

Primers were constructed using the Primer-BLAST online server, according to the sequence of the FASN gene for ovine (GenBank accession numbers NC_019468.2). The primer sequences with a product length of 325 bp used to be as follows: FASN,exo2-F: 5′ CAGATGGAGGAGGGGGATCA 3′ and FASN,exo2-R: 5′ GTCCACAGAAGCGGTGA GAA 3′. This PCR experiment consisted of the following steps: 5 min to initial denaturation, 30 cycles for each 30 sec of 95°C denaturation, 62.9°C annealing, and 72°C elongation, followed by 5 min of 72°C final extension. PCR amplicons were verified for specificity using agarose gel electrophoresis before being subjected to Single-Strand Conformation Polymorphism (SSCP) experiments. Genotyping was conducted according to the protocol described by Imran et al. (2020). The SSCP patterns on the gels were visualized by Byun et al. (2009) using silver staining.

DNA sequencing

All genotypes were sequenced downstream using a BioNeer sequencing machine, in Daejeon, South Korea, with forward and reverse primers. The received chromatograms were edited and aligned using DNA Star, EditSeq. / ClustalW, with the sequences published in the GenBank database taken as a reference. The SnapGene Viewer, ver. 4.0.4 (GSL. Biotech. LLC), and ensemble genome browser 96 (https://asia.ensembl.org/index.html), were used to visualize and check the novelty of the observed mutations of the FASN gene.

In silico analysis

Several computational prediction tools were used to assess the implications of the observed missense variants for mutant protein structure, function, and stability. SIFT, a tool that determines whether substitutions affect the functionality of proteins using

sequence homology and amino acid properties; SNAP2, a tool that predicts mutated protein functionality; and I-Mutant2.0, designed to predict protein stability changes based on single point mutations from protein sequences and structures (Imran et al. 2020). The detected nsSNP was virtually visualized using Phere2, ver 2.0, and PyMol-v1, https://www.schrodinger.com/pymol, predicting FASN's 3D structure prior to and subsequent mutation.

Data analyses

The genetic diversity indices of Awassi sheep, including allele frequency, genotype frequency, observed heterozygosity (H*o*), expected heterozygosity (H*e*), the effective number of alleles (N*e*), and Hardy-Weinberg equilibrium, were analyzed using PopGen32 software, v. 1.31. Statistical analysis was conducted using SPSS v23.0 (IBM, NY, USA). Student's t-tests were used with the following model to examine the effects of genotype on various phenotypic traits:

$$Y_{ij} = \mu + \alpha i + e_{ij}$$

where Y_{ij} is the phenotypic trait, μ is the overall mean, αi is the effect of genotype, i^{th} genotype (i = GG, GA), and e_{ij} is random error assumed to be NID (0, $\sigma^2 e$).

Normality was tested using the Kolmogorov–Smirnov test. Preliminary statistical analyses indicated that age, season, and nutrition were not found to affect the fat traits being investigated, and thus, they were not included in the model.

RESULTS AND DISCUSSION

Genotyping and genetic diversity of the *FASN* gene

The ovine FASN gene's genotyping analysis was conducted using PCR-SSCP and DNA sequencing techniques. The SSCP analysis revealed two GG and GA genotype variations within the DNA samples amplified by the ovine FASN (exon 2) (Figure 1). The genetic analysis revealed that the predominant genotype was GA, with a genotype frequency of 79%. Based on the $\chi 2$ tests (Table 1), FASN gene (exon 2) polymorphism in Awassi sheep was not at Hardy-Weinberg equilibrium at this locus. Higher heterozygosity than expected was observed. For the c.186 G>A SNP, the genetic diversity analysis found higher values for Ne and Higher; this reflected a very high genetic diversity for the FASN gene in ovine populations.

The current genotyping results revealed two genotypes (GG and GA) of the ovine FASN gene (exon 2). Sheep with the GA genotype had lower saturated fatty acids, higher IMF (%), and unsaturated fatty acids. Sequencing reactions confirmed that these two SSCP patterns were positioned within GA genotypes. The FASN gene polymorphism exerts a crucial role in lipogenesis and saturated fatty acid synthesis (Inostroza et al. 2013; Renaville et al. 2018; Malgwi et al. 2022). Several researchers have reported the genetic variations of the FASN gene associated with fatty acid content in livestock. Sztankoova et al. (2018) revealed that the SNP g.257C>T in exon 32 of the FASN gene influences fatty acid composition, in which the genotype TT has higher values for MUFAs and polyunsaturated fatty acids compared to the genotype CC in the Czech sheep population. Besides, the SNP g.17924A>G affects fatty acid content in Nellore cattle (de Souza et al. 2012). Furthermore, the SNP g.16930T>A is associated with higher fatty acid content in yaks (Chu et al. 2015).

Sequence and *in silico* analysis of *FASN* gene

Post-PCR genotyping analysis revealed two different genotypes of Awassi sheep. Sequence analysis of the ovine FASN locus identified seven novel SNPs between the two resolved genotypes and the FASN (exon 2) NCBI reference sequences (GenBank accession numbers NC_019468.2). The pattern of each SNP discovered by sequencing is listed in (Supplementary Table 1). Several SNPs were discovered in FASN (exon 2) reference compared to GG and GA genotypes. Four novel SNPs were identified in genotype GA (G 103 C, G 186 A, C 224 G, T 290 C), compared with two novel SNPs in genotype GG (A 109 C, G 111 C). Both genotypes shared one common SNP (G 287 C) (Supplementary Table 1).

Concerning in silico analysis, all the utilized in silico prediction tools were given deleterious signals for c.186 G>A. The I-Mutant 2 tool additionally confirmed the deleterious effects of c.186 G>A, which predicted a reduction in stability of the FASN protein upon mutation with this SNP (Figure 2 and Supplementary Table 2). This amino acid substitution caused a protein with reduced stability and may have had a detrimental effect on the function of the FASN enzyme, which organizes de novo the synthesis of saturated fatty acids.

To verify whether these SNPs cause the deleterious effect on the encoded FASN protein. Various in silico methods were employed to assess the structural and functional consequences of the novel SNPs. Most in silico analyses referred to the detrimental effects of c.186 G>A SNP on the FASN enzyme. Several nsSNPs can modify enzyme function, alter protein structure, or cause protein interaction disruptions (Patel et al. 2015). As the FASN enzyme is responsible for the biosynthesis of SFA (Otto et al. 2022), it is possible to speculate that the g. 50787138 A>G SNP is responsible for the decreased protein capability to undertake its scheduled task of the FASN enzyme. These cumulative deleterious consequences of c.186 G>A were detected only in the

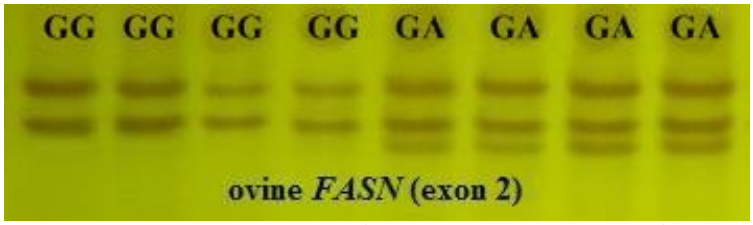

Figure 1. SSCP non-denaturing polyacrylamide gel electrophoresis of ovine *FASN* gene (exon 2) PCR fragments exhibited two SSCP banding patterns in Awassi sheep. SSCP experiment was performed in 10% polyacrylamide gels (37.5:1) at 200 V for 4 h

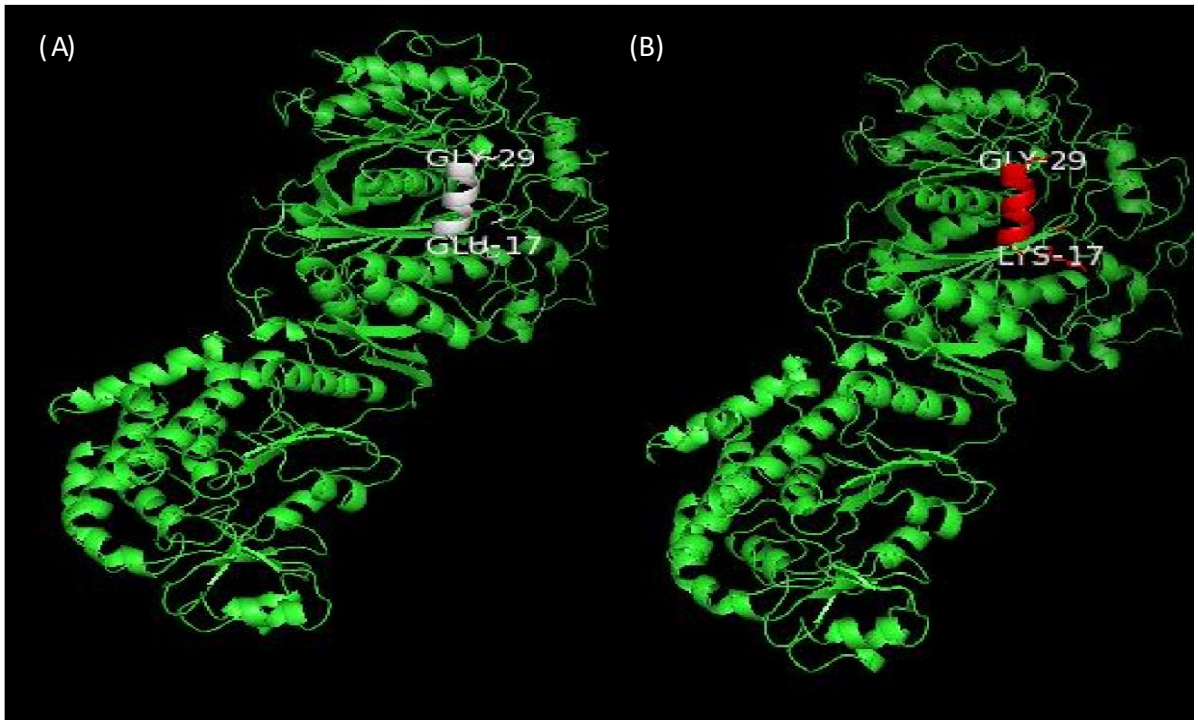

Figure 2. Virtual 3-D structure of ovine FASN. A) Reference type protein (Before mutation), B) mutant protein (in GA genotype)

GA genotype, making them more favored in unsaturated fatty acids content than the GG genotype. This result concord with the study by Bartoň et al. (2021) revealed the association between the FASN gene variant and unsaturated fatty acid content in diverse cattle breeds. Moreover, Otto et al. (2022) demonstrated that g. 50787138 A>G SNP in the FASN gene were associated with a healthier longissimus dorsi fatty acid composition.

FASN (exon 2) gene polymorphism with intramuscular fat content and fatty acid composition

Association analysis for the fatty acid composition in the longissimus dorsi muscle and FASN (exon 2) genotypes is shown in Table (2). A significant association of the FASN (GA) genotypes was observed for the highest content of IMF (%), and lowest content of myristic acid of SFA, total SFA, and with unsaturated fatty acid in which the GA genotype had the highest content of ricinoleic, oleic, total MUFA, PUFA including α-linoleic, and α-linolenic compare to the GG genotypes.

Concerning association analysis, the FASN gene was related to fatty acid content. Compared to the GG genotype, the GA genotype had significantly ($P \leq 0.05$) higher quantities of unsaturated fatty acids and less saturated fatty acids. Fatty acid synthase enzyme participated in de novo lipogenesis in livestock (Bartoň et al. 2021; Otto et al. 2022). A similar study also showed a significant association between FASN levels and fatty acid content, primarily due to FASN polymorphism, resulting in higher mitochondrial oxidation of fatty acids (Mahmoud et al. 2016). Add to that, g. 17924A>G SNP of the FASN gene in Friesian cows is revealed to be associated with the fatty acids composition (Inostroza et al. 2013). Another study showed that three SNPs in the FASN gene are associated with cattle growth and carcass characteristics (de Souza et al. 2012). As a result of the present study, the longissimus dorsi (LD) muscle fatty acid profile is determined concerning the polymorphisms studied, and the results could be used for sheep flock selection: the GA genotype is highly promising for human consumption due to the polymorphism of the FASN gene.

Table 1. Genetic diversity parameters for the *FASN* gene in Awassi breeds.

Genotype frequencies (n)		Allele frequencies		Ho	He	Ne	PIC	$\chi 2$
GA	GG	A	G	0.79	0.48	1.91	0.36	42.01
0.79 (79)	0.21 (21)	0.40	0.60					

Abbreviations= (n) samples number, χ^2 – chi-square, *Ho*= observed heterozygosity, *He*= Expected heterozygosity, *Ne*= effective allele number. All Chi-square tests have one degree of freedom and are within the significance level of P<0.05

Table 2. Relationship between *FASN* (exon 2) gene polymorphism with animal age, carcass weight, IMF (%), and fatty acids composition (% of total FA) in Awassi sheep

Parameters	LSM ± SE		*P* value
	GG	GA	
Animal age at slaughter (year)	2.18 ± 0.07	2.01 ± 0.08	0.53
Carcass weight (kg)	23.32 ± 1.63	22.91 ± 1.04	0.41
IMF (%)	3.06 ± 0.15	5.22 ± 0.35	**0.01**
SFA (% of total FA)			
C8:0	4.09 ± 0.40	3.84 ± 0.12	0.20
C10:0	4.11 ± 0.54	3.88 ± 0.21	0.43
C12:0	1.34 ± 0.16	1.49 ± 0.24	0.16
C14:0	3.73 ± 0.36	2.12 ± 0.19	**0.03**
C16:0	1.90 ± 0.15	1.78 ± 0.27	0.29
C18:0	0.01 ± 0.003	0.03 ± 0.001	0.48
C20:0	1.47 ± 0.11	1.38 ± 0.26	0.13
Total SFA	46.79 ± 2.08	37.41 ± 1.92	**0.03**
MUFA (% of total FA)			
C18:1cis	0.87 ± 0.01	1.18 ± 0.09	**0.01**
C18:1trans	1.52 ± 0.33	1.68 ± 0.26	0.44
C18:1n-9	3.55 ± 0.12	5.74 ± 0.65	**0.01**
C18:1n-9	1.69 ± 0.24	1.52 ± 0.33	0.55
C18:1n-9	0.37 ± 0.08	0.39 ± 0.03	0.13
C18:1n-7	0.11 ± 0.001	0.09 ± 0.002	0.30
Total MUFA	22.20 ± 2.37	29.14 ± 1.83	**0.03**
PUFA (% of total FA)			
C18:2n6	0.65 ± 0.07	1.15 ± 0.23	**0.01**
C18:3n-3	5.01 ± 0.70	4.99 ± 0.64	0.15
C18:3n3	0.41 ± 0.09	0.89 ± 0.02	**0.01**
Total PUFA	12.84 ± 1.75	13.03 ± 2.53	0.20

LSM= Least square mean ±SE standard error, SFA= saturated fatty acid, MUFA= monounsaturated fatty acid, PUFA= polyunsaturated fatty acid. The P-value with statistical significance is indicated in bold numbers

CONCLUSION

The result confirmed that a low SFA content and a high level of MUFA and PUFA characterize the intramuscular fat composition of the GA genotype of Awassi sheep. The c.186 G>A SNP has a considerable negative impact on meat fatty acid composition in Awassi sheep. Therefore, the c.186 G>A SNP in ovine *FASN* gene variation could be useful for assessing carcass traits. Moreover, it may be effective for improving the future direct selection of animals with a healthier fatty acid content, thus improving meat quality.

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Immunity and Behaviour of Lambs Born from Ewes Fed a Flushing Diet Containing EPA and DHA

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ABSTRAK

Nurlatifah A, Khotijah L, Arifiantini RI, Maidin MS, Astuti DA, Herdis. 2023. Imunitas dan kelangsungan hidup anak domba dari induk yang diberi ransum flushing mengandung EPA dan DHA . JITV 28(3):159-168. DOI:http://dx.doi.org/10.14334/jitv.v28.i3. 3110.

Nutrisi berperan penting dalam daya tahan tubuh dan status imunitas anak domba dari periode neonatal hingga sapih. Penelitian ini bertujuan untuk mengevaluasi efek konsentrat *flushing* mengandung *docosahexaenoic acid* (DHA) and *eicosapentaenoic acid* (EPA) asal minyak lemuru pada imunitas dan daya tahan tubuh anak domba. Dua puluh delapan anak domba neonatal dibagi kedalam empat perlakuan bergantung pada pakan yang dikonsumsi oleh induknya. Empat perlakuan meliputi : konsentrat kontrol (P1), konsentrat *flushing* mengandung 6% minyak sawit (P2), konsentrat *flushing* mengandung 3% minyak sawit dan 3 % minyak lemuru (P3), dan konsentrat flushing mengandung 6% minyak lemuru (P4). Domba diberi rumput gajah dan konsentrat dengan rasio 30:70% berdasarkan bahan kering. Induk dan anak domba ditempatkan pada kandang yang sama hingga usia sapih 2 bulan. Parameter yang diamati pada induk domba meliputi immunoglobulin G (Ig G) kolostrum. Parameter yang diamati pada anak domba adalah Ig G darah. Tingkah laku anak neonatal, respon fisiologis, hematologi saat dilahirkan dan saat sapih. Hasil menunjukkan anak dari domba yang diberi konsentrat P4, P3, P2 nyata (P<0,05) lebih singkat waktu berdirinya. Leukosit P1 saat sapih, nyata (P<0,05) lebih tinggi dari P2 dan P3. Leukosit P1 sama tinggi dengan P4. Perlakuan tidak berpengaruh nyata (P>0,05) pada Ig G dan respon fisiologis. Penelitian ini menyimpulkan, konsentrat *flushing* 6% minyak lemuru mengandung DHA dan EPA dua kali kebutuhan yang diberikan pada induk domba dapat mempercepat waktu berdiri pertama anak domba.

Kata Kunci: *Flushing*, Imunitas, Immunoglobulin, Minyak Lemuru

ABSTRACT

Nurlatifah A, Khotijah L, Arifiantini RI, Maidin MS, Astuti DA, Herdis. 2023. Immunity and survival rate of Lamb born from ewe fed flushing diet containing EPA and DHA. JITV 28(3):159-168. DOI:http://dx.doi.org/10.14334/jitv.v28.i3. 3110.

Nutrition can influence lamb survival and the immune status during the neonatal phase until weaning. This study aimed to investigate the effect of flushing of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from Lemuru fish oil in the Ewe's diet on lamb immunity and survival. Twenty-eight neonatal lambs were divided into four treatments according to Ewe's diet. Four treatments: control concentrate (P1), flushing concentrate with 6% palm oil (P2), flushing concentrate with 3% lemuru oil and 3% palm oil (P3), and flushing concentrate with 6% lemuru oil (P4). The Ewe were fed Napier grass and concentrated in a 30:70% ratio based on dry matter. Ewe and their lambs remained together until weaning at about two months of age. The parameter observed in ewes was colostrum Immunoglobulin G (IgG). The parameters in the Lamb are blood Ig G, neonatal behavior of the Lamb, physiological response, and hematology of the Lamb at birth and weaning. The results showed that feeding P4, P3, and P2 to the Ewe resulted in a shorter latency to stand-in lamb (P<0.05). P1 has higher leukocytes (P<0.05) during weaning than P2 and P3. P1 has the same leukocyte as P4. Treatment has a non-significant effect (P>0.05) on Ig G and physiological response. In conclusion, administering 6% Lemuru oil containing EPA and DHA twice can shorten the latency to stand in newborn lambs.

Key Words: Flushing, Immunity, Immunoglobulin, Lamb Survival, Lemuru Oil

INTRODUCTION

Feeding the Ewe has a significant impact on preventing lamb mortality. Ideally, maternal immunity should be transferred to the Lamb in utero. However, placental barriers in ruminants may not allow IgG transmission (Ke et al. 2021). Therefore, the Immune status of the Lamb depends on passive transfer from

colostrum content. Diet affects colostrum composition (Banchero et al. 2015) and lamb behavior (Ahmadzadeh et al. 2020). Shorter latency to rise and suckle is very important for lamb survival. It is associated with colostrum consumption to maintain thermoregulation, transfer passive immune media from the dam, and strengthen the lamb-maternal relationship (Dwyer et al., 2016).

Several studies have been conducted to improve lamb survival. Polyunsaturated fatty acid (PUFA) in the ratio of ewes during lactation could improve milk quality for lamb survival. Ewes fed PUFA from a fish meal-enriched diet had eicosapentaenoic acid and docosahexaenoic acid in colostrum and milk compared to those fed the control diet (Coleman et al. 2018). Flushing ration with PUFA-rich fish oil increases the preovulatory follicle and ovulation rate with an increase in the kidding rate (Mahla et al. 2017).

Long-chain fatty acids Polyunsaturated fatty acids (PUFAs), such as arachidonic acid (C20: 4 (n-6)) and docosahexaenoic acid (C22: 6 (n3)), are fatty acids required for tissue development, synaptic transmission, and influence brain development. Neonatal behavior can be influenced by PUFA intake in Ewe's diet, especially docosahexaenoic acid (DHA3); 20: 06 (n-3) (Roque-Jiménez et al. 2021). The survival rate of newborn lambs can be increased by adding DHA from algae to the ratio of ewes (Pickard et al. 2008). In addition, diets high in EPA and DHA increase the concentration of EPA and DHA fatty acids in muscle tissue in growing lambs (Ferreira et al. 2014). In addition, feeding DHA and EPA during gestation may be a strategy for maintaining the immune system during gestation and for immune system development in lambs (Roque-Jiménez et al. 2021; Veshkini et al. 2020)

Lemuru fish oil is a by-product containing n-3 polyunsaturated fatty acids (PUFA) 13.70% EPA and 22.47% DHA. However, DHA and EPA effect in the ewe ration in late gestation and the lambing period has not been recognized with an apparent effect on lamb survival and immunity. Therefore, this study aimed to investigate the effects of a flushing diet in the ration of ewes on colostrum quality (Ig G), neonatal behavior, physiological response, and hematology.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Nutrition for draught animals, Department of Nutrition and feed technology, Faculty of Animal Science IPB University. Approval for the current study was by the Animal Care and Use Committee (ACUC) at IPB University No. 119-2018 IPB

Primiparous ewes and lambs born from each Ewe were used for this experiment and divided into four treatments. Each treatment consisted of five ewes. Twenty-eight lambs were divided into four treatments depending on the Ewe. Each Lamb was examined from birth to two months of age. The ewes and their Lamb were kept in individual cages and allowed to suckle from birth until weaning at two months of age.

Ration

The experimental ewes were fed a total mixed ration at 3.5% body weight with a forage-to-concentrate ratio of 30:70 on a dry matter basis. Treatments with a flushing concentrate with different oil sources for ewes are as follows: control (P1), flushing concentrate with 6% palm oil (P2), flushing concentrate with 3% lemuru oil and 3% palm oil (P3), and flushing concentrate with 6% lemuru oil (P4). The nutrient content in P1 was adjusted to meet the nutrient requirements of a pregnant ewe according to NRC (2007), while P2, P3, and P4 are flushing concentrates with isoprotein and isoenergy. The EPA and DHA contents in the flushing concentrate are adjusted to the requirement for linolenic acid in the ration given by Pudelkewicz et al. (1968), which is 0.5%. The EPA and DHA levels of P3 flushing rations were 0.47%, while the P4 ration was 0.94%. The flushing concentrate was administered to the ewes twice, two weeks before, two weeks after mating, and two weeks before and two weeks after parturition; outside this period, the ewes received the control ration.

Measurement in the ewe

The nutrient consumption of Ewe after lambing was measured from lambing day until 14 d after parturition; the animals were given P1, P2, P3, and P4. Daily nutritional consumption of concentrate and forage was calculated by removing residual feed from the offered one daily. After getting the amount of feed consumed, multiply by each feed's chemical content.

Colostrum Immunoglobulin G (IgG) content was measured from colostrum collected 24 hours after lambing. ELISA and KIT measured the colostral IgG concentrations for ovine immunoglobulin G (Bioenzy Catalog no: BZ-08199100-EB).

Measurement in the lamb

Plasma immunoglobulin G (IgG) measured from blood samples with anticoagulant was collected from the jugular vein of lambs after seven days. ELISA and KIT measured plasma IgG concentrations for ovine immunoglobulin G (Bioenzy Catalog no: BZ-08199100-EB).

Observation of Lamb's Behavior began at birth and ended at successful emergence. The observation was recorded with a digital camera. The time and frequency

Table 1 Composition and nutrient content flushing concentrate (100% dry matter basis)*

Feed Ingredients	Treatment			
	P1	P2	P3	P4
	----------%----------			
Soybean meal	17.14	28.57	28.57	28.57
Pollard	43.57	29.26	29.26	29.26
Dried cassava	30.00	26.86	26.86	26.86
Lemuru oil	-	-	3	6
Palm oil	-	6	3	-
Molasses	7.14	7.14	7.14	7.14
CaCO3	0.71	0.71	0.71	0.71
Premix	0.71	0.71	0.71	0.71
NaCl	0.71	0.71	0.71	0.71
Nutrient content	----------%----------			
Crude protein	14.39	17.50	17.87	17.49
Crude fat	1.22	7.84	7.61	7.42
Crude fiber	9.92	9.47	9.12	9.25
NFE	63.33	54.15	54.22	54.44
TDN	71.34	73.16	73.24	72.93
EPA and DHA	-	-	0.67	1.34
Palmitic Acid	-	2.61	1.30	-
Ca	0.73	0.82	0.82	0.82
P	0.65	0.56	0.56	0.56

*Result of Nutrition and Feed Technology Laboratory, IPB Univesity (2019) P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. TDN= total digestible nutrient; calculation results Wardeh (1981) TDN= 2.6407 + (0.6964 x %CP) + (1.2159 x %Fat) – (0.1043 x %fiber) + (0.9194 x %NFE)

of observed lamb behavior are: a. Latency to standing (minutes) is the time it takes an infant from birth to standard standing b. Latency to suck (minutes) is the time it takes for an infant to go from birth to successfully sucking milk (Fonsêca et al. 2014)

Physiological responses such as rectal temperature, respiratory rate, and heart rate are measured from days 50 to 65. Measurements are taken three times a day at 07.00 a.m., 12.00 p.m., and 05.00 p.m. The condition of the cage in the morning is 24.13±0.55℃ and relative humidity (RH) 90±5.19%; during the day, the temperature is 30.50 ±0.36℃ and RH 65.55±6.42%, and in the afternoon, the temperature is 29.13±1.67 ℃ and RH 71±8.18%.

Blood hematology was measured at 7 and 56 days of age. Blood samples were collected from a jugular vein in the morning with a 1 mL syringe and collected in a sterile tube containing EDTA anticoagulants. Blood hematology parameters such as hematocrit, hemoglobin, total erythrocytes, and total leukocytes were analyzed according to standard procedures (Zhu et al. 2017).

Experimental design and data analysis

A completely randomized design of experiments (CRD) was used for the study. The data obtained were statistically tested (ANOVA), followed by the Duncan test using SAS ver 19.0.1.

RESULTS AND DISCUSSION

During the flushing period after lambing, treatment did not affect the total dry matter consumption, crude fiber consumption, Nitrogen Free Extract consumption (NFE), and Total Digestible Nutrients (TDN). Crude

protein and fat consumption were significantly highest in treatment P2 and lowest in P1. P3 and P4 were the same with treatment P2. A significant difference in fat and protein consumption occurs due to the different fat and protein content in flushing concentrate compared to the control. Higher fat and protein consumption aligns with a higher tendency of total dry matter. Dry matter consumption, protein consumption, and TDN in this study fulfilled the nutrient needed for Ewe in the early lactating period with single or twin lambs. Based on NRC (2007), the nutritional requirements for lactating Ewe with BW of 40 kg was 720-930 g/day TDN and 156-224 g/day crude protein.

Treatment had no effect (P>0.05) on the concentration of immunoglobulin (IgG) in the colostrum and blood plasma of the lambs. Immunoglobulin data from ewe colostrum and Lamb blood plasma are shown in Table 2. Immunoglobulin G (IgG) is an anti-infective component in blood, colostrum, and milk that contain glycoproteins that dissipate environmental pathogens by binding or encapsulating barriers (Balan et al. 2019). In this study, a high-fat concentrate containing DHA and EPA had no significant effect on immunoglobulin in the colostrum of ewes. This result aligns with another study that reported that feeding EPA and DHA has no significant effect on lambs' colostral IgG and serum IgG concentration (Moreno-Indias et al. 2020). Colostrum synthesis depends on the diet consumed by Ewe during gestation (Banchero et al. 2015). However, the transfer of immunoglobulin did not influence the diet. High-energy feeding harms IgG concentration. An increase in prolactin regulates IgG transfer from blood to colostrum. In contrast, another study reported that feeding positively affects colostrum production and IgG transfer because high nutrient consumption increases colostrum production, affecting the total amount of IgG absorbed by the Lamb. No significant effect means that high-fat content in flushing ration does not decrease IgG concentration in the colostrum. In this study, immunoglobulin concentration ranged from 0.05mg/mL to 0.014 mg/mL. Colostrum is collected 24 hours after lambing. In agreement with another report, low IgG concentration states that colostral IgG concentration decreases rapidly with time (approximately 3.3 mg/mL/h) and drops to zero mg/mL after 24 hours post-lambing (Hinde & Woodhouse 2019).

There is no significant difference in IgG concentration in lamb plasma, as immunoglobulin concentration in colostrum is the same in all treatments. Transfer of Immunoglobulin from Ewe to Lamb by passive transfer. Usually, immunity from the Ewe is transferred in utero to the fetus, born with body immunity. However, in ruminants, there are placental barriers. Therefore, the transfer of immunoglobulin from Ewe to Lamb depends on colostrum (Ke et al. 2021). In this study, immunoglobulin concentration in lambs at 7 days of age is 0.42-0.67 mg/mL. This concentration may lead to increased abomasal secretion and proteolytic activity of intestinal cells and reduce the efficiency of IgG absorption after birth. The ruminant small intestine cell can absorb IgG and effectively transfer it to the blood only 24 hours after birth (Alves et al. 2015).

The behavior of newborn lambs in terms of survival and latency to stand was significantly (P<0.05) faster in treatments P2, P3, and P4 than in controls (P1). Treatment had no significant effect (P>0.05) on latency to suckling (Table 3). Latency to stand, or the time required for a lamb to stand after birth, is related to the survival and growth rate of the Lamb. The shorter time to stand, the greater the chance for the Lamb to avoid cold stress (Kenyon et al. 2019). Latency to standing is significantly shorter in lambs from ewes-fed flushing diets compared to controls. This result is consistent with the report by (Pickard et al. 2008) that adding DHA and EPA to the ration of ewes at the end of gestation can increase survival and influence neonatal behavior.

Neonatal behavior can be influenced by birth weight, sex, litter size, and nutritional status of the dam. Single-born lambs stand up faster than twins (da Porciuncula et al. 2021). This study shows that Lamb from sheep treated with a flushing diet tends to be born in the twin type but requires a shorter latency to stand up than control animals, which tend to be born singles; this is likely due to the fact that the behavior of newborn lambs from sheep fed with the flushing diet is influenced by the added length of the PUFA chain in the sheep diet, especially DHA and EPA (Gulliver et al. 2012). EPA and DHA are essential fatty acids for brain tissue development, synaptic transmission, and retinal development (Duttaroy & Basak 2020). DHA supplementation during pregnancy may positively affect brain cell membrane development, including the cerebellum, which controls locomotor development (Pickard et al. 2008).

Shorter latency to stand in Lamb from Ewe gave lemuru oil as a DHA and EPA source in line with another study on the Lamb (Encinias et al. 2004). The researchers reported an increase in lamb survival fed with higher fat compared to low-fat diets. Adding fat can increase brown adipose tissue's (BAT) thermogenic capacity responsible for nonshivering thermoregulation. Right after birth is a critical time for a newborn lamb because it is associated with heat loss to maintain homeotherm before digested colostrum. The newborn Lamb must metabolize energy reserves in brown fat tissue and increase muscular activity by shivering (Plush et al. 2016).

Lamb is born with fewer energy reserves, so it is crucial to find udders and consume milk as quickly as possible (Agenbag et al. 2021). Latency to suckle or the time needed for a lamb right after birth to suckle to its dam in this study was not significant. Latency to suckle

Table 2. Nutrient consumption of Ewe after lambing

Parameters Consumption	Treatment			
	P1	P2	P3	P4
Dry matter (g/h/d)	1180.53±286.05	1276.30±154.56	1131.02±310.49	946.39±143.17
Crude protein ((g/h/d)	152.29±35.05^{b}	218.83±27.32^{a}	192.46±57.56^{ab}	156.82±20.27^{ab}
Crude fat ((g/h/d)	18.58±4.94^{c}	92.75±11.77^{a}	77.47±23.87^{b}	62.72±7.72^{ab}
Crude fiber ((g/h/d)	170.82±47.05	195.99±23.73	175.38±43.56	148.50±28.45
NFE ((g/h/d)	715.97±170.18	769.03±93.60	676.41±189.08	686.07±139.41
TDN ((g/h/d)	811.72±193.50	1002.40±122.70	877.32±249.48	727.70±102.86

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4 flushing concentrate 6% lemuru oil. ^{a,b,c}, different superscript letters on the same line are significantly different (P<0.05)

Table 2. Effect of flushing concentrate containing EPA and DHA into immunoglobulin in colostrum and blood plasma of lambs

Parameters	Treatment			
	P1	P2	P3	P4
IgG in Colostrum (mg/mL)	0.06±0.07	0.05±0.06	0.05±0.04	0.14±0.22
IgG in Lamb's blood plasma (mg/ML)	0.67±0.44	0.65±0.41	0.42±0.29	0.53±0.43

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,c}, different superscript letters on the same line are significantly different (P<0.05)

Table 3. Lamb behavior born from Ewe fed a flushing diet

Parameters	Treatment			
	P1	P2	P3	P4
Latency to stand (minute)	51.99±58.60^{b}	21.78±18.63^{a}	15.16±16.36^{a}	7.33±6.27^{a}
Latency to suckle (minute)	82.13±53.54	78.27±44.43	43.20±35.96	35.57±31.55

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,c}, different superscript letters on the same line are significantly different (P<0.05)

is also related to the time of successful standing. Latency to suckle tends to be shorter in Lamb from Ewe fed a flushing diet. Shorter latency to stand means that it can provide nutrients, especially to maintain thermoregulation, passive immune media transfer from the parent, and strengthen the relationship between Lamb and Ewe that can affect the survival rate (Dwyer et al. 2016).

Treatments did not significantly affect (P>0.05) lambs' rectal temperature, heart rate, and breath frequency. Physiological responses to Lamb are presented in Table 4. Maintaining normal physiological responses is important because it can support the body's thermoregulation under normal circumstances(Mota-Rojas et al. 2021). The average heart rate of lambs in the morning, day, and afternoon during the study was still within the average heart rate range in sheep (Seixas et al. 2021). The usual range of heart rate is 70-80 times per minute. The result in this study obtained lower than those reported by (Astuti et al. 2019; Fazio et al. 2016) at the age of 30 days; the average heart rate frequency was 150 per times minute. This difference might be due to the age at which physiological response data collection was started in Lamb, aged 50 days. There is a tendency to decrease the heart rate frequency daily compared to the Lamb at birth (Fazio et al. 2016).

The results of the respiration rate in this study are the same as the report of Astuti et al. (2019) and Fazio et al. (2016) in goats and sheep. In Lamb, respiratory frequency is higher than in adult sheep because Lamb has a more significant proportion of body surface area per body weight than adult sheep. Therefore the respiratory rate is higher (Koether et al. 2015). The respiratory rate of a lamb tends to decrease over time. The decrease in respiratory rate from first birth to 30 days ranges from 20 times per minute^{-1} (Fazio et al. 2016).

The rectal temperature of the lamb range within the normal range reported by Seixas et al. (2021) states that

the normal range of average rectal temperature is 38.3-39.9ºC. Normal rectal temperature indicates that the Lamb is in good health. The rectal temperatures in this study are consistent with those reported in Lamb (Astuti et al. 2019; Fazio et al. 2016). The temperature of lambs aged 1-30 days ranges from 39.1 to 39.8℃ under normal conditions. The rectal temperature of a newborn lamb fluctuates until 30 days of age, probably due to two factors: Thermoregulation in Lamb is not very specific, or the mechanism of thermoregulation is still under development (Aleksiev 2009). In this study, the normal physiological response of Lamb suggests that the ewe diet treatment is still able to maintain the thermoregulation of the body in good condition, even though the temperature conditions during the daytime environment may cause increased stress.

Treatment given to the Ewe did not affect (P>0.05) hematocrit values, hemoglobin levels, erythrocytes count, or leukocyte count in the lambs' blood at birth. The hematological data of the seven-day-old lambs are shown in Table 5. Hematological measurements provide essential information about the health status. This study's total number of erythrocyte lamb ranged from 9.46 to 11.24x10^6 mm. The number of erythrocytes Lamb in this study is still in the normal range of 9-15x10^6 mm (Al-Jbory & Al-Samarai 2016). Erythrocytes or red blood cells bind and transport oxygen to all body tissues (Glenn & Armstrong 2019). Normal erythrocytes in this study mean that the Lamb born from an ewe with a flushing ration has a normal and healthy red blood cell.

The lamb blood's hemoglobin value (Hb) in this study ranged from 10.60 to 11.64 g/dL, within the normal range. Normal hemoglobin levels range from 8-11 g/dL (Rahman et al. 2018). Hemoglobin functions to bind oxygen in the blood consisting of porphyrin, Fe, glycine and methyl, propionyl, and vinyl side groups (Barupala et al. 2016). The average hemoglobin level in this study means that feeding the Ewe with flushing can maintain the hemoglobin content and the capacity to transport oxygen in the blood of born lambs at normal levels.

Hematocrit is the percentage of blood cells in the total blood volume (Watson & Maughan 2014). The mean hematocrit of Lamb in this study was in the normal range of 29.28% to 32.00%. The normal hematocrit in sheep is 23% to 37%% (Rahman et al. 2018); this indicates that flushing rations fed to the Ewe can improve the hematocrit level of the Lamb at birth under normal conditions.

Leukocytes are active units in the body's defense system and act against antigens or foreign substances entering the body, prevent infection and phagocytosis, and produce or distribute antibodies as part of the immune response—the low total leukocytes in lambs correlated with a high risk for disease attack (Etim 2015). Leukocyte counts in the study ranged from 4.85 to 6.51x10^3 mm. These results indicate that leukocytes are within the normal range. Normal leukocyte counts in sheep range from 4-12 x10^3 mm (Al-Jbory & Al-Samarai, 2016). This result means that Lamb born from Ewe fed a flushing diet has excellent and healthy immunity.

Treatment had a significant effect (P<0.05) on the leukocytes of weaning Lamb. Leukocyte levels were highest in controls compared with P2 and P3. P4 treatment was not different from the control and P2 and P3. Treatment had no significant effect (P>0.05) on hematocrit levels, hemoglobin content, and erythrocyte counts in the blood of lambs. The hematological data of Lamb sat weaning age are shown in Table 6.

Treatment significantly affected the total leukocyte count in the weaned lambs' blood. Leukocytes are blood components that play a role in maintaining the body's immune system, which is tasked with destroying foreign

Table 4. The physiological response of the Lamb

Parameters	Measurement Time	Treatment			
		P1	P2	P3	P4
Heart rate (time per minute)	Morning	81.50±16.26	80.80±9.96	87.03±12.24	94.10±13.97
	Day	87.50±6.36	83.08±20.24	87.37±6.10	105.67±4.93
	Afternoon	63.50±10.60	77.73±15.37	84.00±11.75	91.20±10.32
Respiratory rate (times per minute)	Morning	51.16±0.23	53.50±7.38	56.13±7.74	48.06±5.88
	Day	56.50±7.77	58.11±5.37	55.20±5.54	63.26±6.19
	Afternoon	49.50±2.12	57.44±6.23	55.23±4.63	48.73±4.52
Rectal temperature (℃)	Morning	39.14±0.41	39.19±0.36	39.13±0.28	39.36±0.21
	Day	39.40±0.07	39.53±0.21	39.64±0.17	39.75±0.17
	Afternoon	38.97±0.45	39.33±0.27	39.41±0.07	39.52±0.22

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,c}, different superscript letters on the same line are significantly different (P<0.05)

Table 5. Hematology of lamb blood at seven days of age

Parameters	Treatment			
	P1	P2	P3	P4
Erythrocytes (10^6 mm)	10.33±2.07	9.46±2.18	9.70±1.41	11.24±2.17
Hemoglobin (g/dL^{-1})	11.48±1.90	10.60±1.01	10.91±1.10	11.64±1.26
Hematocrit (%)	29.28±4.34	29.83±2.71	27.85±2.47	32.00±5.16
Leukocytes (10^3 mm)	6.51±2.11	4.85±1.41	4.93±1.19	5.56±2.22

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,} different superscript letters on the same line significantly different (P<0.05).

Table 6. Hematology of lamb blood at weaning age

Parameters	Treatment			
	P1	P2	P3	P4
Erythrocytes (10^6 mm)	8.41±0.07	8.83±1.94	8.47±0.44	9.73±0.95
Hemoglobin (g/dL)	12.85±0.63	11.56±2.40	11.82±0.74	12.88±0.62
Hematocrit (%)	32.50±2.12	31.00±1.89	31.02±2.36	32.80±1.64
Leukocytes (10^3 mm)	11.80±0.28^a	6.92±2.99^b	6.41±0.73^b	10.54±3.95^{ab}

Source: Peter et al. (2002)

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,} different superscript letters on the same line significantly different (P<0.05).

bodies that are harmful to the body (Etim 2015). Although treatment P1 has higher leukocyte levels, the result is within the normal range, according to (Al-Jbory & Al-Samarai 2016).

Average leukocyte values in weaning sheep are 4-12 10^3 mm. Average leukocyte values can be interpreted as a sign that there are no non-specific disorders in the Lamb's body and its immune status is in order. The difference in leukocyte levels in the P1 treatment may be related to the type of birth control.

A high number of leukocytes in the P4 treatment indicates that although the type of birth in P4 is more likely to be twins with a lower birth weight than the control (Nurlatifah et al. 2022), it can still produce a lamb with the same total leukocytes as the control, this is consistent with the finding report that differences in the hematological profiles of lambs can be influenced by birth type and birth weight (Ashour et al. 2015).

Supplementation of 6% lemuru oil containing EPA and DHA twice from maintenance to the Ewe may produce a lamb with a good immune system at weaning age. The effect of n-3 PUFA on immunity is thought to be related to changes in the production of eicosanoids such as PGE2. PGE2 may inhibit lymphocyte proliferation. The decrease of PGE2 by EPA metabolism may increase lymphocyte proliferation. The tendency for leukocyte proliferation occurs with an increase in EPA and DHA (Peterson et al. 1998). Fish oil supplementation altered the composition of lymphocyte fatty acids, increasing ω 3-3/ω-6 from 0.18 to 0.62. Supplementation with DHA-rich fish oil results in a 40% increase in lymphocyte proliferative capacity, which depends on Concavallin A as determined by thymidine incorporation. DHA-rich fish oil also causes an increase in phagocytosis of neutrophils and monocyte (Gorjao et al. 2006).

The hematocrit, hemoglobin, total leukocytes, and total erythrocytes obtained in this study are within the range of average values, according to (Al-Jbory & Al-Samarai 2016; Rahman et al. 2018). Hematocrit values, hemoglobin, and erythrocytes are not significantly different, suggesting that feeding flushing feed to the Ewe can maintain normal hematology in lambs born to weaning. The number of hematocrits, hemoglobin, leukocytes, and erythrocytes increased significantly with the lambs' age, which agrees with the result of this study. There is improvement in total leukocyte, hemoglobin, and hematocrit values at the weaning age compared to the neonatal period (Antunović et al. 2012).

CONCLUSION

In conclusion, feeding 6% Lemuru oil containing EPA and DHA on ewe diet during early and late gestation until two weeks post-partum could improve the behavior of newborn lambs by shortening latency to stand from birth to normal standing. Feeding 6% lemuru fish oil showed no significant effects on lamb IgG levels but did alter the total leukocytes of Lamb at weaning. The mechanism of omega-3 effect on lamb immunity needs further investigation.

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Immunity and Behaviour of Lambs Born from Ewes Fed a Flushing Diet Containing EPA and DHA

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ABSTRAK

Nurlatifah A, Khotijah L, Arifiantini RI, Maidin MS, Astuti DA, Herdis. 2023. Imunitas dan kelangsungan hidup anak domba dari induk yang diberi ransum flushing mengandung EPA dan DHA . JITV 28(3):159-168. DOI:http://dx.doi.org/10.14334/jitv.v28.i3. 3110.

Nutrisi berperan penting dalam daya tahan tubuh dan status imunitas anak domba dari periode neonatal hingga sapih. Penelitian ini bertujuan untuk mengevaluasi efek konsentrat *flushing* mengandung *docosahexaenoic acid* (DHA) and *eicosapentaenoic acid* (EPA) asal minyak lemuru pada imunitas dan daya tahan tubuh anak domba. Dua puluh delapan anak domba neonatal dibagi kedalam empat perlakuan bergantung pada pakan yang dikonsumsi oleh induknya. Empat perlakuan meliputi : konsentrat kontrol (P1), konsentrat *flushing* mengandung 6% minyak sawit (P2), konsentrat *flushing* mengandung 3% minyak sawit dan 3 % minyak lemuru (P3), dan konsentrat flushing mengandung 6% minyak lemuru (P4). Domba diberi rumput gajah dan konsentrat dengan rasio 30:70% berdasarkan bahan kering. Induk dan anak domba ditempatkan pada kandang yang sama hingga usia sapih 2 bulan. Parameter yang diamati pada induk domba meliputi immunoglobulin G (Ig G) kolostrum. Parameter yang diamati pada anak domba adalah Ig G darah. Tingkah laku anak neonatal, respon fisiologis, hematologi saat dilahirkan dan saat sapih. Hasil menunjukkan anak dari domba yang diberi konsentrat P4, P3, P2 nyata ($P<0,05$) lebih singkat waktu berdirinya. Leukosit P1 saat sapih, nyata ($P<0,05$) lebih tinggi dari P2 dan P3. Leukosit P1 sama tinggi dengan P4. Perlakuan tidak berpengaruh nyata ($P>0,05$) pada Ig G dan respon fisiologis. Penelitian ini menyimpulkan, konsentrat *flushing* 6% minyak lemuru mengandung DHA dan EPA dua kali kebutuhan yang diberikan pada induk domba dapat mempercepat waktu berdiri pertama anak domba.

Kata Kunci: *Flushing*, Imunitas, Immunoglobulin, Minyak Lemuru

ABSTRACT

Nurlatifah A, Khotijah L, Arifiantini RI, Maidin MS, Astuti DA, Herdis. 2023. Immunity and survival rate of Lamb born from ewe fed flushing diet containing EPA and DHA. JITV 28(3):159-168. DOI:http://dx.doi.org/10.14334/jitv.v28.i3. 3110.

Nutrition can influence lamb survival and the immune status during the neonatal phase until weaning. This study aimed to investigate the effect of flushing of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from Lemuru fish oil in the Ewe's diet on lamb immunity and survival. Twenty-eight neonatal lambs were divided into four treatments according to Ewe's diet. Four treatments: control concentrate (P1), flushing concentrate with 6% palm oil (P2), flushing concentrate with 3% lemuru oil and 3% palm oil (P3), and flushing concentrate with 6% lemuru oil (P4). The Ewe were fed Napier grass and concentrated in a 30:70% ratio based on dry matter. Ewe and their lambs remained together until weaning at about two months of age. The parameter observed in ewes was colostrum Immunoglobulin G (IgG). The parameters in the Lamb are blood Ig G, neonatal behavior of the Lamb, physiological response, and hematology of the Lamb at birth and weaning. The results showed that feeding P4, P3, and P2 to the Ewe resulted in a shorter latency to stand-in lamb ($P<0.05$). P1 has higher leukocytes ($P<0.05$) during weaning than P2 and P3. P1 has the same leukocyte as P4. Treatment has a non-significant effect ($P>0.05$) on Ig G and physiological response. In conclusion, administering 6% Lemuru oil containing EPA and DHA twice can shorten the latency to stand in newborn lambs.

Key Words: Flushing, Immunity, Immunoglobulin, Lamb Survival, Lemuru Oil

INTRODUCTION

Feeding the Ewe has a significant impact on preventing lamb mortality. Ideally, maternal immunity should be transferred to the Lamb in utero. However, placental barriers in ruminants may not allow IgG transmission (Ke et al. 2021). Therefore, the Immune status of the Lamb depends on passive transfer from colostrum content. Diet affects colostrum composition (Banchero et al. 2015) and lamb behavior (Ahmadzadeh

et al. 2020). Shorter latency to rise and suckle is very important for lamb survival. It is associated with colostrum consumption to maintain thermoregulation, transfer passive immune media from the dam, and strengthen the lamb-maternal relationship (Dwyer et al. 2016).

Several studies have been conducted to improve lamb survival. Polyunsaturated fatty acid (PUFA) in the ratio of ewes during lactation could improve milk quality for lamb survival. Ewes fed PUFA from a fish meal-enriched diet had eicosapentaenoic acid and docosahexaenoic acid in colostrum and milk compared to those fed the control diet (Coleman et al. 2018). Flushing ration with PUFA-rich fish oil increases the preovulatory follicle and ovulation rate with an increase in the kidding rate (Mahla et al. 2017).

Long-chain fatty acids Polyunsaturated fatty acids (PUFAs), such as arachidonic acid (C20: 4 (n-6)) and docosahexaenoic acid (C22: 6 (n3)), are fatty acids required for tissue development, synaptic transmission, and influence brain development. Neonatal behavior can be influenced by PUFA intake in Ewe's diet, especially docosahexaenoic acid (DHA3); 20: 06 (n-3) (Roque-Jiménez et al. 2021). The survival rate of newborn lambs can be increased by adding DHA from algae to the ratio of ewes (Pickard et al. 2008). In addition, diets high in EPA and DHA increase the concentration of EPA and DHA fatty acids in muscle tissue in growing lambs (Ferreira et al. 2014). In addition, feeding DHA and EPA during gestation may be a strategy for maintaining the immune system during gestation and for immune system development in lambs (Roque-Jiménez et al. 2021; Veshkini et al. 2020)

Lemuru fish oil is a by-product containing n-3 polyunsaturated fatty acids (PUFA) 13.70% EPA and 22.47% DHA. However, DHA and EPA effect in the ewe ration in late gestation and the lambing period has not been recognized with an apparent effect on lamb survival and immunity. Therefore, this study aimed to investigate the effects of a flushing diet in the ration of ewes on colostrum quality (Ig G), neonatal behavior, physiological response, and hematology.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Nutrition for draught animals, Department of Nutrition and feed technology, Faculty of Animal Science IPB University. Approval for the current study was by the Animal Care and Use Committee (ACUC) at IPB University No. 119-2018 IPB

Primiparous ewes and lambs born from each Ewe were used for this experiment and divided into four treatments. Each treatment consisted of five ewes. Twenty-eight lambs were divided into four treatments depending on the Ewe. Each Lamb was examined from birth to two months of age. The ewes and their Lamb were kept in individual cages and allowed to suckle from birth until weaning at two months of age.

Ration

The experimental ewes were fed a total mixed ration at 3.5% body weight with a forage-to-concentrate ratio of 30:70 on a dry matter basis. Treatments with a flushing concentrate with different oil sources for ewes are as follows: control (P1), flushing concentrate with 6% palm oil (P2), flushing concentrate with 3% lemuru oil and 3% palm oil (P3), and flushing concentrate with 6% lemuru oil (P4). The nutrient content in P1 was adjusted to meet the nutrient requirements of a pregnant ewe according to NRC (2007), while P2, P3, and P4 are flushing concentrates with isoprotein and isoenergy. The EPA and DHA contents in the flushing concentrate are adjusted to the requirement for linolenic acid in the ration given by Pudelkewicz et al. (1968), which is 0.5%. The EPA and DHA levels of P3 flushing rations were 0.47%, while the P4 ration was 0.94%. The flushing concentrate was administered to the ewes twice, two weeks before, two weeks after mating, and two weeks before and two weeks after parturition; outside this period, the ewes received the control ration.

Measurement in the ewe

The nutrient consumption of Ewe after lambing was measured from lambing day until 14 d after parturition; the animals were given P1, P2, P3, and P4. Daily nutritional consumption of concentrate and forage was calculated by removing residual feed from the offered one daily. After getting the amount of feed consumed, multiply by each feed's chemical content.

Colostrum Immunoglobulin G (IgG) content was measured from colostrum collected 24 hours after lambing. ELISA and KIT measured the colostral IgG concentrations for ovine immunoglobulin G (Bioenzy Catalog no: BZ-08199100-EB).

Measurement in the lamb

Plasma immunoglobulin G (IgG) measured from blood samples with anticoagulant was collected from the jugular vein of lambs after seven days. ELISA and KIT measured plasma IgG concentrations for ovine immunoglobulin G (Bioenzy Catalog no: BZ-08199100-EB).

Observation of Lamb's Behavior began at birth and ended at successful emergence. The observation was recorded with a digital camera. The time and frequency

Table 1 Composition and nutrient content flushing concentrate (100% dry matter basis)*

Feed Ingredients	Treatment			
	P1	P2	P3	P4
	----------%----------			
Soybean meal	17.14	28.57	28.57	28.57
Pollard	43.57	29.26	29.26	29.26
Dried cassava	30.00	26.86	26.86	26.86
Lemuru oil	-	-	3	6
Palm oil	-	6	3	-
Molasses	7.14	7.14	7.14	7.14
CaCO3	0.71	0.71	0.71	0.71
Premix	0.71	0.71	0.71	0.71
NaCl	0.71	0.71	0.71	0.71
Nutrient content	----------%----------			
Crude protein	14.39	17.50	17.87	17.49
Crude fat	1.22	7.84	7.61	7.42
Crude fiber	9.92	9.47	9.12	9.25
NFE	63.33	54.15	54.22	54.44
TDN	71.34	73.16	73.24	72.93
EPA and DHA	-	-	0.67	1.34
Palmitic Acid	-	2.61	1.30	-
Ca	0.73	0.82	0.82	0.82
P	0.65	0.56	0.56	0.56

*Result of Nutrition and Feed Technology Laboratory, IPB Univesity (2019) P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. TDN= total digestible nutrient; calculation results Wardeh (1981) TDN= 2.6407 + (0.6964 x %CP) + (1.2159 x %Fat) – (0.1043 x %fiber) + (0.9194 x %NFE)

of observed lamb behavior are: a. Latency to standing (minutes) is the time it takes an infant from birth to standard standing b. Latency to suck (minutes) is the time it takes for an infant to go from birth to successfully sucking milk (Fonsêca et al. 2014)

Physiological responses such as rectal temperature, respiratory rate, and heart rate are measured from days 50 to 65. Measurements are taken three times a day at 07.00 a.m., 12.00 p.m., and 05.00 p.m. The condition of the cage in the morning is 24.13±0.55℃ and relative humidity (RH) 90±5.19%; during the day, the temperature is 30.50 ±0.36℃ and RH 65.55±6.42%, and in the afternoon, the temperature is 29.13±1.67℃ and RH 71±8.18%.

Blood hematology was measured at 7 and 56 days of age. Blood samples were collected from a jugular vein in the morning with a 1 mL syringe and collected in a sterile tube containing EDTA anticoagulants. Blood hematology parameters such as hematocrit, hemoglobin, total erythrocytes, and total leukocytes were analyzed according to standard procedures (Zhu et al. 2017).

Experimental design and data analysis

A completely randomized design of experiments (CRD) was used for the study. The data obtained were statistically tested (ANOVA), followed by the Duncan test using SAS ver 19.0.1.

RESULTS AND DISCUSSION

During the flushing period after lambing, treatment did not affect the total dry matter consumption, crude fiber consumption, Nitrogen Free Extract consumption (NFE), and Total Digestible Nutrients (TDN). Crude

protein and fat consumption were significantly highest in treatment P2 and lowest in P1. P3 and P4 were the same with treatment P2. A significant difference in fat and protein consumption occurs due to the different fat and protein content in flushing concentrate compared to the control. Higher fat and protein consumption aligns with a higher tendency of total dry matter. Dry matter consumption, protein consumption, and TDN in this study fulfilled the nutrient needed for Ewe in the early lactating period with single or twin lambs. Based on NRC (2007), the nutritional requirements for lactating Ewe with BW of 40 kg was 720-930 g/day TDN and 156-224 g/day crude protein.

Treatment had no effect (P>0.05) on the concentration of immunoglobulin (IgG) in the colostrum and blood plasma of the lambs. Immunoglobulin data from ewe colostrum and Lamb blood plasma are shown in Table 2. Immunoglobulin G (IgG) is an anti-infective component in blood, colostrum, and milk that contain glycoproteins that dissipate environmental pathogens by binding or encapsulating barriers (Balan et al. 2019). In this study, a high-fat concentrate containing DHA and EPA had no significant effect on immunoglobulin in the colostrum of ewes. This result aligns with another study that reported that feeding EPA and DHA has no significant effect on lambs' colostral IgG and serum IgG concentration (Moreno-Indias et al. 2020). Colostrum synthesis depends on the diet consumed by Ewe during gestation (Banchero et al. 2015). However, the transfer of immunoglobulin did not influence the diet. High-energy feeding harms IgG concentration. An increase in prolactin regulates IgG transfer from blood to colostrum. In contrast, another study reported that feeding positively affects colostrum production and IgG transfer because high nutrient consumption increases colostrum production, affecting the total amount of IgG absorbed by the Lamb. No significant effect means that high-fat content in flushing ration does not decrease IgG concentration in the colostrum. In this study, immunoglobulin concentration ranged from 0.05 mg/mL to 0.014 mg/mL. Colostrum is collected 24 hours after lambing. In agreement with another report, low IgG concentration states that colostral IgG concentration decreases rapidly with time (approximately 3.3 mg/mL/h) and drops to zero mg/mL after 24 hours post-lambing (Hinde & Woodhouse 2019).

There is no significant difference in IgG concentration in lamb plasma, as immunoglobulin concentration in colostrum is the same in all treatments. Transfer of Immunoglobulin from Ewe to Lamb by passive transfer. Usually, immunity from the Ewe is transferred in utero to the fetus, born with body immunity. However, in ruminants, there are placental barriers. Therefore, the transfer of immunoglobulin from Ewe to Lamb depends on colostrum (Ke et al. 2021). In this study, immunoglobulin concentration in lambs at 7 days of age is 0.42-0.67 mg/mL. This concentration may lead to increased abomasal secretion and proteolytic activity of intestinal cells and reduce the efficiency of IgG absorption after birth. The ruminant small intestine cell can absorb IgG and effectively transfer it to the blood only 24 hours after birth (Alves et al. 2015).

The behavior of newborn lambs in terms of survival and latency to stand was significantly (P<0.05) faster in treatments P2, P3, and P4 than in controls (P1). Treatment had no significant effect (P>0.05) on latency to suckling (Table 3). Latency to stand, or the time required for a lamb to stand after birth, is related to the survival and growth rate of the Lamb. The shorter time to stand, the greater the chance for the Lamb to avoid cold stress (Kenyon et al. 2019). Latency to standing is significantly shorter in lambs from ewes-fed flushing diets compared to controls. This result is consistent with the report by (Pickard et al. 2008) that adding DHA and EPA to the ration of ewes at the end of gestation can increase survival and influence neonatal behavior.

Neonatal behavior can be influenced by birth weight, sex, litter size, and nutritional status of the dam. Single-born lambs stand up faster than twins (da Porciuncula et al. 2021). This study shows that Lamb from sheep treated with a flushing diet tends to be born in the twin type but requires a shorter latency to stand up than control animals, which tend to be born singles; this is likely due to the fact that the behavior of newborn lambs from sheep fed with the flushing diet is influenced by the added length of the PUFA chain in the sheep diet, especially DHA and EPA (Gulliver et al. 2012). EPA and DHA are essential fatty acids for brain tissue development, synaptic transmission, and retinal development (Duttaroy & Basak 2020). DHA supplementation during pregnancy may positively affect brain cell membrane development, including the cerebellum, which controls locomotor development (Pickard et al. 2008).

Shorter latency to stand in Lamb from Ewe gave lemuru oil as a DHA and EPA source in line with another study on the Lamb (Encinias et al. 2004). The researchers reported an increase in lamb survival fed with higher fat compared to low-fat diets. Adding fat can increase brown adipose tissue's (BAT) thermogenic capacity responsible for nonshivering thermoregulation. Right after birth is a critical time for a newborn lamb because it is associated with heat loss to maintain homeotherm before digested colostrum. The newborn Lamb must metabolize energy reserves in brown fat tissue and increase muscular activity by shivering (Plush et al. 2016).

Lamb is born with fewer energy reserves, so it is crucial to find udders and consume milk as quickly as possible (Agenbag et al. 2021). Latency to suckle or the time needed for a lamb right after birth to suckle to its dam in this study was not significant. Latency to suckle

Table 2. Nutrient consumption of Ewe after lambing

Parameters Consumption	Treatment			
	P1	P2	P3	P4
Dry matter (g/h/d)	1180.53±286.05	1276.30±154.56	1131.02±310.49	946.39±143.17
Crude protein ((g/h/d)	152.29±35.05[b]	218.83±27.32[a]	192.46±57.56[ab]	156.82±20.27[ab]
Crude fat ((g/h/d)	18.58±4.94[c]	92.75±11.77[a]	77.47±23.87[b]	62.72±7.72[ab]
Crude fiber ((g/h/d)	170.82±47.05	195.99±23.73	175.38±43.56	148.50±28.45
NFE ((g/h/d)	715.97±170.18	769.03±93.60	676.41±189.08	686.07±139.41
TDN ((g/h/d)	811.72±193.50	1002.40±122.70	877.32±249.48	727.70±102.86

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4 flushing concentrate 6% lemuru oil. [a,b,c], different superscript letters on the same line are significantly different (P<0.05)

Table 2. Effect of flushing concentrate containing EPA and DHA into immunoglobulin in colostrum and blood plasma of lambs

Parameters	Treatment			
	P1	P2	P3	P4
IgG in Colostrum (mg/mL)	0.06±0.07	0.05±0.06	0.05±0.04	0.14±0.22
IgG in Lamb's blood plasma (mg/mL)	0.67±0.44	0.65±0.41	0.42±0.29	0.53±0.43

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. [a,b,c], different superscript letters on the same line are significantly different (P<0.05)

Table 3. Lamb behavior born from Ewe fed a flushing diet

Parameters	Treatment			
	P1	P2	P3	P4
Latency to stand (minute)	51.99±58.60[b]	21.78±18.63[a]	15.16±16.36[a]	7.33±6.27[a]
Latency to suckle (minute)	82.13±53.54	78.27±44.43	43.20±35.96	35.57±31.55

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. [a,b,c], different superscript letters on the same line are significantly different (P<0.05)

is also related to the time of successful standing. Latency to suckle tends to be shorter in Lamb from Ewe fed a flushing diet. Shorter latency to stand means that it can provide nutrients, especially to maintain thermoregulation, passive immune media transfer from the parent, and strengthen the relationship between Lamb and Ewe that can affect the survival rate (Dwyer et al. 2016).

Treatments did not significantly affect (P>0.05) lambs' rectal temperature, heart rate, and breath frequency. Physiological responses to Lamb are presented in Table 4. Maintaining normal physiological responses is important because it can support the body's thermoregulation under normal circumstances(Mota-Rojas et al. 2021). The average heart rate of lambs in the morning, day, and afternoon during the study was still within the average heart rate range in sheep (Seixas et al. 2021). The usual range of heart rate is 70-80 times per minute. The result in this study obtained lower than those reported by (Astuti et al. 2019; Fazio et al. 2016) at the age of 30 days; the average heart rate frequency was 150 per times minute. This difference might be due to the age at which physiological response data collection was started in Lamb, aged 50 days. There is a tendency to decrease the heart rate frequency daily compared to the Lamb at birth (Fazio et al. 2016).

The results of the respiration rate in this study are the same as the report of Astuti et al. (2019) and Fazio et al. (2016) in goats and sheep. In Lamb, respiratory frequency is higher than in adult sheep because Lamb has a more significant proportion of body surface area per body weight than adult sheep. Therefore the respiratory rate is higher (Koether et al. 2015). The respiratory rate of a lamb tends to decrease over time. The decrease in respiratory rate from first birth to 30 days ranges from 20 times per minute^{-1} (Fazio et al. 2016).

The rectal temperature of the lamb range within the normal range reported by Seixas et al. (2021) states that the normal range of average rectal temperature is 38.3-39.9ºC. Normal rectal temperature indicates that the

Lamb is in good health. The rectal temperatures in this study are consistent with those reported in Lamb (Astuti et al. 2019; Fazio et al. 2016). The temperature of lambs aged 1-30 days ranges from 39.1 to 39.8℃ under normal conditions. The rectal temperature of a newborn lamb fluctuates until 30 days of age, probably due to two factors: Thermoregulation in Lamb is not very specific, or the mechanism of thermoregulation is still under development (Aleksiev 2009). In this study, the normal physiological response of Lamb suggests that the ewe diet treatment is still able to maintain the thermoregulation of the body in good condition, even though the temperature conditions during the daytime environment may cause increased stress.

Treatment given to the Ewe did not affect (P>0.05) hematocrit values, hemoglobin levels, erythrocytes count, or leukocyte count in the lambs' blood at birth. The hematological data of the seven-day-old lambs are shown in Table 5. Hematological measurements provide essential information about the health status. This study's total number of erythrocyte lamb ranged from 9.46 to 11.24x10^6/mm^3. The number of erythrocytes Lamb in this study is still in the normal range of 9-15x10^6/mm^3 (Al-Jbory & Al-Samarai 2016). Erythrocytes or red blood cells bind and transport oxygen to all body tissues (Glenn & Armstrong 2019). Normal erythrocytes in this study mean that the Lamb born from an ewe with a flushing ration has a normal and healthy red blood cell.

The lamb blood's hemoglobin value (Hb) in this study ranged from 10.60 to 11.64 g/dL, within the normal range. Normal hemoglobin levels range from 8-11 g/dL (Rahman et al. 2018). Hemoglobin functions to bind oxygen in the blood consisting of porphyrin, Fe, glycine and methyl, propionyl, and vinyl side groups (Barupala et al. 2016). The average hemoglobin level in this study means that feeding the Ewe with flushing can maintain the hemoglobin content and the capacity to transport oxygen in the blood of born lambs at normal levels.

Hematocrit is the percentage of blood cells in the total blood volume (Watson & Maughan 2014). The mean hematocrit of Lamb in this study was in the normal range of 29.28% to 32.00%. The normal hematocrit in sheep is 23% to 37%% (Rahman et al. 2018); this indicates that flushing rations fed to the Ewe can improve the hematocrit level of the Lamb at birth under normal conditions.

Leukocytes are active units in the body's defense system and act against antigens or foreign substances entering the body, prevent infection and phagocytosis, and produce or distribute antibodies as part of the immune response—the low total leukocytes in lambs correlated with a high risk for disease attack (Etim 2015). Leukocyte counts in the study ranged from 4.85 to 6.51x10^3 mm. These results indicate that leukocytes are within the normal range. Normal leukocyte counts in sheep range from 4-12 x10^3/mm^3 (Al-Jbory & Al-Samarai, 2016). This result means that Lamb born from Ewe fed a flushing diet has excellent and healthy immunity.

Treatment had a significant effect (P<0.05) on the leukocytes of weaning Lamb. Leukocyte levels were highest in controls compared with P2 and P3. P4 treatment was not different from the control and P2 and P3. Treatment had no significant effect (P>0.05) on hematocrit levels, hemoglobin content, and erythrocyte counts in the blood of lambs. The hematological data of Lamb sat weaning age are shown in Table 6.

Treatment significantly affected the total leukocyte count in the weaned lambs' blood. Leukocytes are blood components that play a role in maintaining the body's immune system, which is tasked with destroying foreign

Table 4. The physiological response of the Lamb

Parameters	Measurement Time	Treatment			
		P1	P2	P3	P4
Heart rate (time per minute)	Morning	81.50±16.26	80.80±9.96	87.03±12.24	94.10±13.97
	Day	87.50±6.36	83.08±20.24	87.37±6.10	105.67±4.93
	Afternoon	63.50±10.60	77.73±15.37	84.00±11.75	91.20±10.32
Respiratory rate (times per minute)	Morning	51.16±0.23	53.50±7.38	56.13±7.74	48.06±5.88
	Day	56.50±7.77	58.11±5.37	55.20±5.54	63.26±6.19
	Afternoon	49.50±2.12	57.44±6.23	55.23±4.63	48.73±4.52
Rectal temperature (℃)	Morning	39.14±0.41	39.19±0.36	39.13±0.28	39.36±0.21
	Day	39.40±0.07	39.53±0.21	39.64±0.17	39.75±0.17
	Afternoon	38.97±0.45	39.33±0.27	39.41±0.07	39.52±0.22

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,c}, different superscript letters on the same line are significantly different (P<0.05)

Table 5. Hematology of lamb blood at seven days of age

Parameters	Treatment			
	P1	P2	P3	P4
Erythrocytes (10^6 mm)	10.33±2.07	9.46±2.18	9.70±1.41	11.24±2.17
Hemoglobin (g/dL^{-1})	11.48±1.90	10.60±1.01	10.91±1.10	11.64±1.26
Hematocrit (%)	29.28±4.34	29.83±2.71	27.85±2.47	32.00±5.16
Leukocytes (10^3 mm)	6.51±2.11	4.85±1.41	4.93±1.19	5.56±2.22

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,} different superscript letters on the same line significantly different (P<0.05).

Table 6. Hematology of lamb blood at weaning age

Parameters	Treatment			
	P1	P2	P3	P4
Erythrocytes (10^6 mm)	8.41±0.07	8.83±1.94	8.47±0.44	9.73±0.95
Hemoglobin (g/dL)	12.85±0.63	11.56±2.40	11.82±0.74	12.88±0.62
Hematocrit (%)	32.50±2.12	31.00±1.89	31.02±2.36	32.80±1.64
Leukocytes (10^3 mm)	11.80±0.28^a	6.92±2.99^b	6.41±0.73^b	10.54±3.95^{ab}

Source: Peter et al. (2002)

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,} different superscript letters on the same line significantly different (P<0.05).

bodies that are harmful to the body (Etim 2015). Although treatment P1 has higher leukocyte levels, the result is within the normal range, according to (Al-Jbory & Al-Samarai 2016).

Average leukocyte values in weaning sheep are 4-12 10^3 mm. Average leukocyte values can be interpreted as a sign that there are no non-specific disorders in the Lamb's body and its immune status is in order. The difference in leukocyte levels in the P1 treatment may be related to the type of birth control.

A high number of leukocytes in the P4 treatment indicates that although the type of birth in P4 is more likely to be twins with a lower birth weight than the control (Nurlatifah et al. 2022), it can still produce a lamb with the same total leukocytes as the control, this is consistent with the finding report that differences in the hematological profiles of lambs can be influenced by birth type and birth weight (Ashour et al. 2015).

Supplementation of 6% lemuru oil containing EPA and DHA twice from maintenance to the Ewe may produce a lamb with a good immune system at weaning age. The effect of n-3 PUFA on immunity is thought to be related to changes in the production of eicosanoids such as PGE2. PGE2 may inhibit lymphocyte proliferation. The decrease of PGE2 by EPA metabolism may increase lymphocyte proliferation. The tendency for leukocyte proliferation occurs with an increase in EPA and DHA (Peterson et al. 1998). Fish oil supplementation altered the composition of lymphocyte fatty acids, increasing ω 3-3/ω-6 from 0.18 to 0.62. Supplementation with DHA-rich fish oil results in a 40% increase in lymphocyte proliferative capacity, which depends on Concavallin A as determined by thymidine incorporation. DHA-rich fish oil also causes an increase in phagocytosis of neutrophils and monocyte (Gorjao et al. 2006).

The hematocrit, hemoglobin, total leukocytes, and total erythrocytes obtained in this study are within the range of average values, according to (Al-Jbory & Al-Samarai 2016; Rahman et al. 2018). Hematocrit values, hemoglobin, and erythrocytes are not significantly different, suggesting that feeding flushing feed to the Ewe can maintain normal hematology in lambs born to weaning. The number of hematocrits, hemoglobin, leukocytes, and erythrocytes increased significantly with the lambs' age, which agrees with the result of this study. There is improvement in total leukocyte, hemoglobin, and hematocrit values at the weaning age compared to the neonatal period (Antunović et al. 2012).

CONCLUSION

In conclusion, feeding 6% Lemuru oil containing EPA and DHA on ewe diet during early and late gestation until two weeks post-partum could improve the behavior of newborn lambs by shortening latency to stand from birth to normal standing. Feeding 6% lemuru fish oil showed no significant effects on lamb IgG levels but did alter the total leukocytes of Lamb at weaning. The mechanism of omega-3 effect on lamb immunity needs further investigation.

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Polymorphism of Melanocortin-4 Receptor Gene and Its Association with Growth Traits in Bali Cattle

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ABSTRAK

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Melanocortin-4 receptor (MC4R) merupakan gen yang turut mengontrol sifat-sifat pertumbuhan. Gen MC4R masuk dalam *leptin-melanocortin pathway* yang bertugas mengatur bobot badan. Penelitian sebelumnya telah banyak berhasil dalam mengidentifikasi keragaman genetik gen MC4R yang terkait dengan sifat pertumbuhan. Namun demikian, penelitian serupa pada sapi Bali masih sangat terbatas. Penelitian ini bertujuan untuk mengetahui *marker* SNP gen MC4R dan pengaruhnya terhadap bobot badan lahir, bobot badan sapih, lingkar dada sapih, tinggi pundak sapih, panjang badan sapih, bobot badan *yearling*, lingkar dada *yearling*, tinggi pundak *yearling*, dan panjang badan *yearling* pada sapi Bali (n=43). Genotip gen MC4R diidentifikasi menggunakan metode *sequencing* dan disejajarkan melalui program BioEdit v.7.2.5. Studi asosiasi pengaruh genotip terhadap sifat pertumbuhan dianalisis berdasarkan prosedur GLM dan DMRT pada program RStudio v.2022.02. Empat SNP berhasil teridentifikasi pada wilayah ekson: g.355G>T, g.394C>T, g.463G>A, dan g.682G>A. Berdasarkan uji *Chi-square*, populasi memenuhi kesetimbangan HWE (p>0.05). Asosiasi genotip-fenotip berdasarkan SNP menunjukkan hasil yang tidak signifikan (p>0.05) dimana lokus g.682G>A (AG) memiliki nilai WB (104.33±17.15 kg), WCG (112.83±3.66 kg), WBL (89.50±3.94 kg), YB (124.17±22.44 kg), YCG (120.50±5.50 kg), dan YBL (92.83±4.31 kg) lebih tinggi dibandingkan genotip lainnya. Asosiasi berdasarkan tipe haplotip menunjukkan hasil yang signifikan (P<0.05) pada BB, dimana haplotip 2 memiliki BB tertinggi (21.17±0.75 kg). Kesimpulannya, *marker* SNP dalam penelitian ini bersifat polimorfik akan tetapi tidak mempengaruhi sifat pertumbuhan pada sapi Bali.

Kata Kunci: Sapi Bali, Keragaman Genetik, Sifat Pertumbuhan, Gen MC4R, *Single Nucleotide Polymorphism*

ABSTRACT

Kusminanto RY, Hazimi FA, Ceri VPA, Panjono, Hartatik T. 2023. Polymorphism of melanocortin-4 receptor gene and its association with growth traits in Bali cattle. JITV 28(3):177-186. DOI:http://dx.doi.org/10.14334/jitv.v28i3.3163.

The melanocortin-4 receptor (MC4R) is a gene that controls growth traits. This gene is embedded in the leptin-melanocortin pathway and regulates body weight. Previous studies have successfully identified the genetic diversity of the MC4R associated with growth traits. However, studies on Bali cattle are limited. This study aimed to identify the SNP markers of the MC4R gene and its effect on birth body weight, weaning body weight, weaning chest girth, weaning withers height, weaning body length, yearling body weight, yearling chest girth, yearling withers height, and yearling body length in Bali cattle (n=43). The MC4R genotype was identified by sequencing and aligned using BioEdit v.7.2.5. The association between genotype and growth traits was analyzed using the GLM procedure and DMRT in RStudio program v.2022.02. Four SNPs were identified in the exon region: g.355G>T, g.394C>T, g.463G>A, and g.682G>A. Based on the Chi-square test, the population was fitted with HWE (p>0.05). Genotype-phenotype association based on SNPs showed no significant result (p>0.05) where the g.682G>A (AG) locus had values of WB (104.33±17.15 kg), WCG (112.83±3.66 kg), WBL (89.50±3.94 kg), YB (124.17±22.44 kg), YCG (120.50±5.50 kg), and YBL (92.83±4.31 kg) higher than the other genotypes. Association based on haplotype type showed significant results (p<0.05) in BB, where haplotype 2 had the highest BB (21.17±0.75 kg). In conclusion, the SNP markers found in this study were polymorphic but did not affect growth traits in Bali cattle.

Key Words: Bali Cattle, Genetic Diversity, Growth Traits, MC4R Gene, Single Nucleotide Polymorphism

INTRODUCTION

Bali cattle *(Bos javanicus)* are known to become the third species of domesticated cattle in addition to *Bos taurus* and *Bos indicus* (Mohamad et al. 2012). Bali cattle have abundant superior traits such as good adaptation to harsh and limited feed resources, tick resistance, a pregnancy rate of up to 88.44% with a birth

rate of 75-85%, a carcass percentage of approximately 53-56%, tick resistance, and low meat fat content (Wawo 2018; Hafid et al. 2019). The increase in meet demand for the market makes the government and stakeholders synergize to improve the genetics of Bali cattle. Improving meat production to meet sufficient market demand also requires good breeding practices for Bali cattle production. Selection based on the genomic level remains a complex study. Marker Assisted Selection (MAS) is a useful and highly efficient method of modern animal selection (Zhao et al., 2020). Detecting single nucleotide polymorphism (SNP) can represent nucleotide variants that serve as genetic markers. In recent years, genetic markers have been the primary criteria used for selection. Genetic markers can resolve traditional selection limitations, which require a relatively long time (Jakaria et al. 2021). In biotechnology, the genetic marker is a DNA fragment associated with a specific location in the genome to identify parts of the DNA sequence in an unknown DNA pool (Singh et al. 2014). The melanocortin-4 receptor (MC4R) is widely used to detect polymorphism by examining SNP as a candidate genetic marker for assessing growth traits.

The melanocortin-4 receptor gene in cattle is located on chromosome 24 with a length of 1,808 base pairs (bp) and consists of only one exon as a region containing coding sequences (CDS) (Liu et al. 2020). The melanocortin-4 receptor is a peptide produced in the hypothalamus of mammals to control food intake and energy expenditure (Ayers et al., 2018). It is one of the smallest G-protein coupled receptor (GPCR) superfamily members, which increases the intracellular level of cyclic AMP (cAMP) and activates protein kinase A (PKA) (Ju et al. 2018). Mutations in the MC4R gene knockout have been implicated in hypophagia in rats and pigs (You et al. 2016; Hao et al. 2019). Thus, the MC4R gene is the key to regulating satiety, energy expenditure, blood pressure, and growth in the leptin-melanocortin signaling pathways (Kühnen et al., 2018). A previous study successfully confirmed the association between MC4R and growth traits in several species, such as pigs, sheep, cattle, and camels (Saini et al. 2018; Shishay et al. 2019; Liu et al. 2020; Al-sharif et al. 2022).

A previous study successfully identified the genotype-phenotype association of several genes in Bali cattle. The SNP g.10428C>T in the stearoyl-CoA desaturase (SCD) gene is associated with marbling score and intramuscular trait (Alwiyah et al. 2016; Karimah et al. 2021). Several SNP in calpain1 (CAPN1) are associated with the carcass, meat characteristics, and backfat thickness, namely g.3669T>C, g.3854G>A, g.3899C>T, and g.15525G>A (Pratiwi et al. 2016; Dairoh et al. 2021). However, the genotype-phenotype association of the MC4R gene in Bali cattle has yet to be reported. Single nucleotide polymorphism detection of the MC4R gene in Bali cattle is needed to determine the population's genetic diversity. Furthermore, information on SNP has become a valuable tool for identifying genetic markers as a characteristic of each individual. This study aimed to analyze SNP markers of the MC4R gene in Bali cattle to map the association between genotype and growth traits.

MATERIALS AND METHODS

Sample collection

This study involved blood and phenotype data from 12 male and 31 female Bali cattle from the Breeding Centre of Superior Livestock and Forage (BPTU-HPT Denpasar), Bali Province. Cattle were kept in a semi-intensive system and maintained under the same feeding system. Collecting 43 blood samples from Bali cattle was performed through the jugular vein with a minimum volume of 3 ml and kept in an EDTA tube. Phenotype data of body weight and body measurements at birth, weaning, and yearling were obtained from the phenotype data record. The phenotype data used in this study included birth body weight (BB), weaning body weight (WB), weaning chest girth (WCG), weaning withers height (WWH), weaning body length (WBL), yearling body weight (YB), yearling chest girth (YCG), yearling withers height (YWH), and yearling body length (YBL).

DNA extraction and amplification

All molecular analyses (except sequencing) were performed at the Breeding and Genetics Laboratory, Faculty of Animal Science, Universitas Gadjah Mada. DNA extraction of 200 µl whole blood was performed using the Geneaid (gSYNC™ DNA extraction kit, Taiwan) protocol. A primer pair (F: 5'-AATGAACTCTACCCAGCCCC-3'; R: 5'-CAGCAGACAACAAAGACCCC-3') of the MC4R gene, located in the exon region was designed based on the GenBank Acc. no. EU366351.1. Amplification of the 774 bp PCR product was performed using a PCR machine (Peqlab Primus 25). The total volume used in the PCR reaction is 25 µl consisting of 9.5 µl DDW, 12.5 PCR kit, 0.5 µl of each primer, and 2 µl DNA extraction. Amplification of the MC4R PCR product was performed under the following conditions: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation, annealing, and extension at 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 30 s, respectively. The final extension was done at 72 °C for 10 min.

Electrophoresis and DNA sequencing

The PCR products were analyzed by electrophoresis before DNA sequencing. The electrophoresis was performed on agarose gel with a concentration of 0.8%

containing 0.25 µl of Ethidium Bromide (EtBr) using a Mupid-EXU electrophoresis machine at 100 volts for 30 minutes. A 1 kb marker was also added to measure the size of the PCR products. The DNA bands were visualized using a UV transilluminator (UVP TEM-40). In total, 43 PCR products of the MC4R gene were sequenced by 1st BASE Malaysia. The reference sequence (EU366351.1, OL623708-OL623717) and sample sequences were analyzed using the BioEdit v.7.2.5. Finally, representative sequences of each haplotype were submitted to GenBank (Submission ID: 2618554).

Data analysis

The genetic polymorphism parameters of allele frequency, genotype frequency, heterozygosity (H), Polymorphic Information Content (PIC), Hardy-Weinberg Equilibrium (HWE), and the association study of genotype-phenotype were analyzed using the RStudio program. The frequency of allele and genotype were calculated using the formula (Nei & Kumar 2000):

$$X_i = \frac{2n_{ii} + \sum_{i \neq j} n_{ij}}{2N}$$

$$X_{ii} = \frac{n_{ii}}{N}$$

where X_i is the frequency of the allele, X_{ii} is the frequency of the genotype, n_{ii} is the number of individuals with genotype ii, n_{ij} is the number of individuals with genotype ij, and N is the total sample.

The HWE was analyzed using the Chi-square test according to Nei & Kumar (2000) as follows:

$$X^2 = \sum \frac{(O - E)^2}{E}$$

where X^2 is the Chi-square, O is the observed value, and E is the expected value.

The association study of genotype-phenotype for each SNP was calculated in one-way Anova using the RStudio program according to the following statistical general linear model:

$$Y_{ij} = \mu + G_i + E_{ij}$$

Where Y_{ij} is the observation of the phenotype, µ is the overall mean, G_i is the effect of the genotype, and E_{ij} is the random error. All data are described as least square means±standard error of means (LSM±SEM). If the Anova value was significant, further testing was performed using Duncan's Multiple Range Test (DMRT).

Correction factor

The phenotype data record was corrected to the parent's age correction factor (FKUI) to reduce environmental errors. The birth body weight data of females were corrected towards males with a correction factor of 1.07 (USDA). Weaning and yearling body weight data were corrected to 205 and 365 days, respectively, according to Hardjosubroto (1994) as follows:

$$WB_{205} = \left[\frac{WBw - BB}{Age} x205 + BB\right](FKUI)$$

$$YB_{365} = [\frac{YBw - WB}{\Delta t} x160 + WB_{205}$$

where WB_{205} is the corrected weaning body weight, YB_{365} is the corrected yearling body weight, WBw is the weaning body weight when weighing, BB is the birth body weight, age is the age at the time of weaning, YBw is the yearling body weight when weighing, WB is the weaning body weight, and Δt is the period from weaning until yearling weighing. The FKUI of Bali cattle followed Pane (1981) for 5-9 years old (1.00). Body size data were corrected by using the body weight correction factor formula.

RESULTS AND DISCUSSION

DNA amplification and SNP identification

The specific DNA fragment of MC4R was successfully amplified, as indicated by clear DNA bands at 774 bp in the exon region (Figure 1). The DNA sequencing results of 43 samples were used for alignment. In total, four SNP markers with a length of 774 bp from gene target: g.355G>T (GG, TG), g.394C>T (CC, CT), g.463G>A (GG, AG, AA), and g.682G>A (GG, AG) were found in this study by comparing the DNA sequencing results with EU366351.1 and OL623708-OL623717, as the GenBank reference (Figure 2). BioEdit showed that there were differences in the nucleotide positions according to Acc. no. EU366351.1 between the sample and the GenBank reference (OL623708-OL623717), whereas they were the same mutation (Table 1). All SNP markers were in the exon region as coding sequences (CDS) that changed nucleotides to proteins during translation. Three SNP (g.394C>T, g.463G>A, g.682G>A) showed a transition mutation, whereas SNP g.355G>T was a transversion mutation that changed the purine to pyrimidine (Table 2). However, all SNPs in this study were classified as silent mutations; therefore, they did not change the amino acid code.

Biomolecular techniques are based on the identification of genetic markers that affect important. traits such as growth traits. A tool such as SNP genotyping helps investigate marker-trait associations (Bali et al. 2018). Single nucleotide polymorphism can be used as markers to determine allele variation as candidate genes in the selection process. One gene related to growth traits is MC4R. Based on the SNP

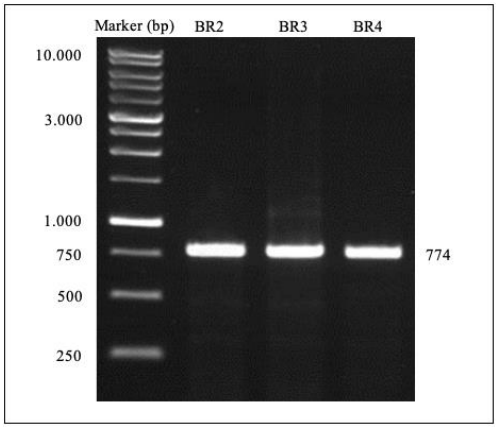

Figure 1. DNA amplification of the MC4R gene on a 1 kb DNA ladder and 0.8% agarose gel. BR2-4: Sample code

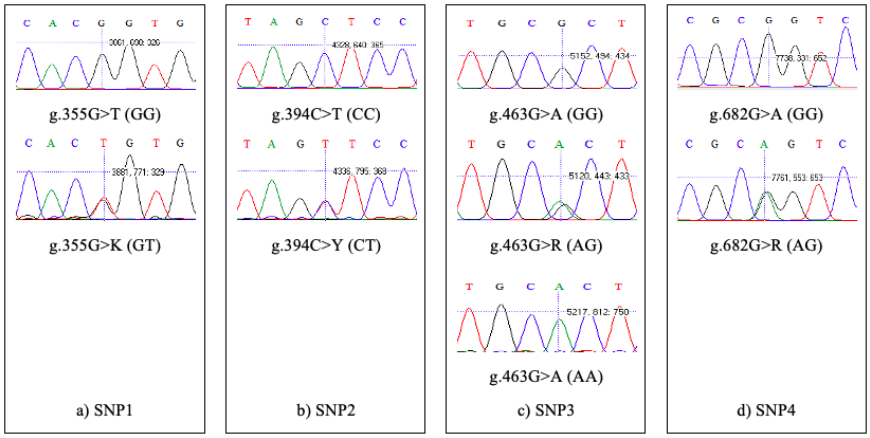

Figure 2. The chromatogram indicates the genotype of four SNP markers in the MC4R gene

Table 1. Nucleotide position between samples and GenBank references

GenBank[a]	GenBank[b]	This study
g.631G>T	g.731G>T	g.355G>T
g.670C>T	g.770C>T	g.394C>T
g.739G>A	g.839G>A	g.463G>A
g.958G>A	g.1058G>A	g.682G>A

[a]EU366351.1; [b](Perdana and Hartatik 2022)

Table 2. Types of the MC4R gene mutations in each SNP

SNP	Mutation	Amino acid change
g.355G>T	Transversion - Silent	ACG (Thr) > ACT (Thr)
g.394C>T	Transition - Silent	AGC (Ser) > AGT (Ser)
g.463G>A	Transition - Silent	GCG (Ala) > GCA (Ala)
g.682G>A	Transition - Silent	GCG (Ala) > GCA (Ala)

genotyping of the MC4R gene (774 bp), this study successfully identified the population balance, including genetic diversity, HWE, and genotype association with growth traits in Bali cattle. Our data showed that the four SNP markers (g.355G>T, g.394C>T, g.463G>A, and g.682G>A) of MC4R were the same as those in a previous study (Perdana and Hartatik 2022). Other studies that have reported MC4R SNP markers in cattle include g.19C>A, g.20A>T, g.83T>C, g.85A>G, g.128G>A, g.129A>G, g.192T>G, g.193A>T,

g.293C>G, g.709G>A, g.927C>T, g.1069C>G, g.1133C>G, g.1343C>A, and g.1786C>T (Seong et al. 2012; Du et al. 2013; Lee et al. 2013; Fathoni et al. 2020; Liu et al. 2020; Utomo et al. 2021).

Genetic diversity

Information about genetic diversity, including the frequency of genotypes and alleles, H, PIC, and HWE of the MC4R gene in Bali cattle, are presented in Table 3. Three of the four loci contained two genotypes: g.355G>T (GG, TG), g.394C>T (CC, CT), and g.682G>A (GG, AG). The locus g.463G>A has three kinds of genotypes: AA, AG, and GG. The loci (g.355G>T and g.394C>T) had the same genotype and allele compositions. As shown in Table 3, the genotypes GG (g.355G>T), CC (g.394C>T), AG (g.463G>A), and GG (g.682G>A) were higher than others. Based on this study's four SNP markers of MC4R, seven haplotype types (five heterozygous and two homozygous) were formed with various percentages (Table 4). Haplotype 1 (GenBank Acc. no. OP376529) had the highest frequency (53%) compared to others. The frequencies of haplotype 2 (Acc. no. OP376530) and haplotype 3 (Acc. no. OP376531) were 14%. Haplotype 4 (Acc. no. OP376532) and haplotype 5 (Acc. no. OP376533) at the same frequency (7%). The lowest frequency (2%) for haplotype 6 (Acc. no. OP376534) and haplotype 7 (Acc. no. OP376535).

Genetic diversity is essential for the adaptation and survival of populations to avoid extinction. For humans, it is important to study population genetics to achieve preservation goals and to perform good breeding practices to maximize genetic potential. There are several methods to calculate genetic diversity, such as heterozygosity (H), runs of homozygosity (ROH), Wright's F-statistic (F_{st}), linkage disequilibrium (LD), and effective population size (N_e) (Al-Mamun et al. 2015). In this study, we used heterozygosity to estim ate genetic diversity. As shown in Table 3, homozygous genotypes (GG and CC) in g.355G>T, g.394C>T, and g.682G>A dominated the genotype (0.95, 0.95 and 0.86), and allele frequency (0.98, 0.98, and 0.93), respectively. In contrast, the heterozygous genotype (AG) at locus g.463A>G (0.63) was higher than that in the homozygous, whereas allele G was still higher than A. A previous study on Bali cattle in BPTU-HPT Denpasar reported that the GG genotype and G allele were the most prevalent (Fahira et al. 2022). According to the genotype and allele information of four loci, the MC4R gene of Bali cattle in BPTU-HPT Denpasar was polymorphic (less than 0.99) (Volkandari et al. 2013). The gene becomes monomorphic if the allele exceeds 0.99 (Putra et al. 2021).

The heterozygosity score of the three SNP markers ranged from 0.05-0.13 with a PIC of 0.04-0.12. The PIC value is closely related to the H score, which depends on the number of alleles; this indicates that the three SNP markers had low polymorphism (PIC<0.25). On the other hand, the SNP g.463G>A, including three genotypes (GG, AG, AA), has almost the same allele (G= 0.55; A= 0.45), while the heterozygosity and PIC values were 0.50 and 0.37, respectively. This g.463G>A SNP marker indicates that the locus was in moderate polymorphism ($0.25 \leq PIC \leq 0.5$) (Botstein et al. 1980).

Table 3. Genetic diversity of the MC4R gene in Bali cattle

Locus	Genotype	N	X_{ii}	X_i	H	PIC	HWE (P-value)
g.355G>T	GG	41	0.95	G: 0.98	0.05	0.04	1
	TG	2	0.05	T: 0.02			
	TT	0	0				
g.394C>T	CC	41	0.95	C: 0.98	0.05	0.04	1
	TC	2	0.05	T: 0.02			
	TT	0	0				
g.463G>A	GG	10	0.23	G : 0.55	0.50	0.37	0.07
	AG	27	0.63	A: 0.45			
	AA	6	0.14				
g.682G>A	GG	37	0.86	G : 0.93	0.13	0.12	1
	AG	6	0.14	A: 0.07			
	AA	0	0				

N= Number of individu; X_{ii}= Frequency of genotype; X_i= Frequency of allele; H= Heterozigosity; PIC= Polymorphic Information Content; HWE= Hardy-Weinberg Equilibrium; α= 0.05

Table 4. Haplotype and frequency of the MC4R gene in Bali cattle

Hap	N	g.355G>T	g.394C>T	g.463G>A	g.682G>A	Frequency (%)	Allele
1	23	G	C	R	G	53	Heterozygous
2	6	G	C	A	G	14	Homozygous
3	6	G	C	G	G	14	Homozygous
4	3	G	C	G	R	7	Heterozygous
5	3	G	C	R	R	7	Heterozygous
6	1	K	Y	G	G	2	Heterozygous
7	1	K	Y	R	G	2	Heterozygous

Hap= Haplotype; N= Number of individu; G, C, A= Homozygous; K= Heterozygous of GT; Y= Heterozygous of CT; R= Heterozygous of AG

A higher PIC value indicates a higher degree of polymorphism (Shan et al., 2020). The low genetic diversity observed in the Bali cattle may be due to selection within a limited area and population. Selection in a limited population can lead to a decrease or loss of one of the minor genes or genetic drift. Mutations and genetic drifts control genetic diversity in populations. Mutation can increase genetic variation, but genetic drift tends to reduce it (Teixeira and Huber 2021). Genetic drift is the leading cause of genetic diversity loss in several cattle breeds, including Canadienne, Milking Shorthorn, Brown Swiss, Guernsey, and Ayrshire (Melka et al. 2013). Genetic drift mainly occurs because of the small effective population size that accumulates over non-founder generations. The Chi-square (X^2) test showed that the genotype distributions of the Bali cattle population were in HWE (P>0.05). The HWE law states that the genotype and allele frequencies will always be the same from generation to generation during random mating (Lachance 2016).

Association of genotype with growth traits

This study analyzes the genotype-phenotype using two analysis approaches based on SNP markers and haplotypes. The values of the genotype-phenotype associations based on the four MC4R SNP markers are presented in Table 5. Statistical analysis of MC4R genotypes and growth traits based on SNP markers revealed no significant association between the four SNP markers (P>0.05). Therefore, the heterozygous (g.682G>A) has a higher body weight and body size than the other three SNP markers, namely, WB (104.33±17.15 kg), WCG (112.83±3.66 kg), WBL (89.50±3.94 kg), YB (124.17±22.44 kg), YCG (120.50±5.50 kg), and YBL (92.83±4.31 kg). The second approach used in this study was based on the haplotype types produced. One-way ANOVA from five haplotypes of body weight and body size showed significant differences in BB (p<0.05) (Table 6). The highest BB was in haplotype 2 (GCAG) with 21.17±0.75 kg. Despite no significant (P>0.05), haplotype type 4 (GCGR) produced the higher body weight and body size of Bali cattle, namely, WB (109.67±21.94 kg), WCG (114.00±5.20 kg), WWH (94.67±4.62 kg), WBL (89.67±2.89 kg), YB (131.33±26.56 kg), YCG (121.33±7.51 kg), and YBL (93.67±1.15 kg).

The genotype-phenotype association study on growth traits of Bali cattle to determine the correlation between SNP markers, body weight, and body size was done. None of the SNP markers in the present study showed significant associations. A previous study found an association between SNP markers and growth traits. However, some did not. Association studies between MYF5 and PLAG1 with body weight and size in Bali cattle from BPTU-HPT Denpasar have shown no significant (Saputra et al. 2020; Fahira et al. 2022). In contrast, the previous studies reported that each genotype has a different effect on the economic feature. For example, the CC genotype in SNP g.1069C>G of Chinese and Korean cattle has better economic features of backfat thickness than the GG genotype (Seong et al. 2012). This study's highest body weight and size scores were for heterozygous SNP marker g.682G>A (AG). The mutation occurring in SNP g.682G>A may affect the increase in body weight and body size at the age of weaning and yearling. As shown in Table 5 and Table 6, the mean birth body weight (BB) was 19.74 kg, and the highest BB was located in haplotype type 2 (21.17±0.75 kg) (P<0.05). This finding was higher than a previous study in which the BB was 17.8 kg (Kaswati et al. 2013). It is conducted that the selection program in BPTU-HPT Denpasar successfully increased the BB of Bali cattle. However, the significant association between haplotype type and BB cannot yet be used as a genetic marker because BB is still highly dependent on the mother (Sulistiyoningtiyas et al. 2017). Selection based on high birth weight is not recommended because of the difficulty of parent-bearing.

Table 5. Association of growth traits with four SNP markers of the MC4R gene in Bali cattle

Locus	X_{ii}	N	BB (kg)	WB (kg)	WCG (cm)	WWH (cm)	WBL (cm)	YB (kg)	YCG (cm)	YWH (cm)	YBL (cm)
g.355G>T	GG	41	19.78±1.96	96.71±13.30	108.76±7.59	92.12±4.61	86.71±5.56	115.61±18.14	117.34±6.97	95.95±4.23	91.19±5.11
	TG	2	19.00±2.83	96.00±4.24	111.00±1.41	92.00±7.07	86.00±4.24	112.00±0.00	119.50±0.71	96.50±2.12	90.00±1.41
	P-value		0.589	0.941	0.682	0.972	0.861	0.782	0.667	0.857	0.746
g.394C>T	CC	41	19.78±1.96	96.71±13.30	108.76±7.59	92.12±4.61	86.71±5.56	115.61±18.14	117.34±6.97	95.95±4.23	91.19±5.11
	TC	2	19.00±2.83	96.00±4.24	111.00±1.41	92.00±7.07	86.00±4.24	112.00±0.00	119.50±0.71	96.50±2.12	90.00±1.41
	P-value		0.589	0.941	0.682	0.972	0.861	0.782	0.667	0.857	0.746
g.463G>A	GG	10	20.20±2.10	100.20±16.38	110.70±5.38	94.50±4.45	87.70±5.75	122.40±22.58	119.10±6.72	97.00±4.50	91.70±5.35
	AG	27	19.26±1.95	96.15±12.21	108.96±8.07	91.18±4.60	86.78±5.54	113.37±15.55	117.26±6.79	95.41±4.18	91.18±5.10
	AA	6	21.17±0.75	93.17±10.81	105.33±7.12	92.33±4.27	84.50±4.85	113.17±18.36	115.50±7.69	96.83±3.49	90.00±4.56
	P-value		0.067	0.555	0.382	0.154	0.53	0.375	0.589	0.514	0.81
g.682G>A	GG	37	19.65±1.99	95.43±12.02	108.22±7.71	91.95±4.80	86.22±5.58	114.03±16.78	116.95±6.94	95.95±4.29	90.86±5.10
	AG	6	20.33±1.86	104.33±17.15	112.83±3.66	93.17±3.60	89.50±3.94	124.17±22.44	120.50±5.50	96.17±3.37	92.83±4.31
	P-value		0.482	0.121	0.16	0.555	0.175	0.197	0.241	0.905	0.377

Values are shown as the least squares means±standard error. X_{ii} = Genotype; N = Number of individu; BB = Birth body weight; WB = Weaning body weight; WCG = Weaning chest girth; WWH = Weaning withers height; WBL = Weaning body length; YB = Yearling body weight; YCG = Yearling chest girth; YWH = Yearling withers height; YBL = Yearling body length; $\alpha = 0.05$

Table 6. Association of growth traits with the haplotype of the MC4R gene in Bali cattle

Hap	N	BB (kg)	WB (kg)	WCG (cm)	WWH (cm)	WBL (cm)	YB (kg)	YCG (cm)	YWH (cm)	YBL (cm)
1	23	19.00+1.81^{b}	95.65+12.61	108.48+8.66	91.30+4.87	86.61+5.65	112.96+15.73	116.83+7.18	95.39+4.48	91.17+5.15
2	6	21.17+0.75^{a}	93.17+10.81	105.33+7.12	92.33+4.27	84.50+4.85	113.17+18.36	115.50+7.69	96.83+3.49	90.00+4.56
3	6	20.83+2.32^{a}	96.67+14.54	109.17+5.64	94.00+5.06	86.50+7.20	119.67+23.60	118.00+7.38	97.00+5.25	90.83+6.91
4	3	20.00+0.00a^{b}	109.67+ 21.94	114.00+5.20	94.67+4.62	89.67+2.89	131.33+26.56	121.33+7.51	96.67+4.62	93.67+1.15
5	3	20.67+2.89^{ab}	99.00+13.00	111.67+1.53	91.67+2.08	89.33+5.51	117.00+19.97	119.67+4.16	95.67+2.52	92.00+6.56
P-value		0.049*	0.493	0.553	0.634	0.668	0.549	0.769	0.900	0.900

Values are shown as the least squares means±standard error. Hap = Haplotype; N = number of individu; BB = Birth body weight; WB = Weaning body weight; WCG = Weaning chest girth; WWH = Weaning withers height; WBL = Weaning body length; YB = Yearling body weight; YCG = Yearling chest girth; YWH = Yearling withers height; YBL = Yearling body length; The different superscripts ($^{a, b}$) in the same column denote significant difference ($\alpha = 0.05$). Only 7% or more was used for an association between growth traits and haplotypes

CONCLUSION

In summary, four SNPs in the exon region (774 bp) of MC4R in Bali cattle were successfully identified. The Bali cattle population in this study had low to moderate polymorphism and fit the HWE. Association analysis of the SNP markers did not show significant results. On the other hand, the association analysis for haplotype showed a significant result for BB. However, this study provides information about the genotype of four SNPs that are useful for reference in subsequent studies and breeding methods based on genetic information.

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Embryo Production and Development from Superovulated Donors in Double-Muscled Cattle and Their Crosses

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ABSTRAK

Irma, Rasad SD, Hilmia N, Sumantri C. 2023. Produksi dan perkembangan embrio sapi donor double-muscled dan persilangannya yang disuperovulasi. JITV 28 (3):187-196. DOI:http://dx.doi.org/10.14334/jitv.v28.i3.3148.

Introduksi sapi Belgian Blue ke Indonesia dilakukan untuk meningkatkan keanekaragaman sumber daya genetik ternak. Persilangan Belgian Blue (BB) dengan sapi Peranakan Ongole (PO) dilaksanakan untuk meningkatkan produktivitas sapi lokal. Tujuan penelitian ini yaitu menganalisis respon superovulasi, perkembangan embrio praimplantasi dan kualitas embrio *in vivo* Belgian Blue, Peranakan Ongole dan persilangannya. Sinkronisasi estrus dilakukan secara intravaginal menggunakan progesteron Cue-Mate sebelum inseminasi buatan (IB). Superovulasi menggunakan *Follicle Stimulating Hormone* (FSH) secara intramuskuler dan panen embrio dilakukan secara non-bedah. Penilaian kualitas embrio secara mikroskopis mengacu pada kritera *International Embryo Transfer Society* (IETS). Metode penelitian yang digunakan adalah quasi eksperimental, data dianalisis dengan analisis sidik ragam (analysis of variance). Oosit dan embrio berhasil diperoleh dari semua bangsa donor setelah disuperovulasi. Terdapat perbedaan produksi oosit dan embrio donor Peranakan Ongole dan Belgian Blue (berturut-turut PO dan BB yaitu 11.83±1.91 *vs* 4.86±1.33, $p<0.05$). Terdapat perbedaan *recovery rate* (89.63% *vs* 75.35%) dan tingkat fertilisasi (77.35% *vs* 68.22%) antara PO dengan BB. Tidak terdapat perbedaan antara fase perkembangan dan kualitas embrio, serta proporsi embrio layak transfer antar bangsa donor BB, PO dan persilangannya. Penelitian ini menunjukkan bahwa sapi donor crossbred hasil persilangan Belgian Blue dengan PO memiliki karakteristik produksi, *recovery rate*, tingkat fertilisasi dan embrio degeneratif yang sama dengan bangsa murninya.

Kata Kunci:, Flushing Embrio, *In Vivo*, Pra-implantasi, Sapi, Superovulasi

ABSTRACT

Irma, Rasad SD, Hilmia N, Sumantri C. 2023. Embryo production and development from superovulated donors in double-muscled cattle and their crosses. JITV 28 (3):187-196. DOI:http://dx.doi.org/10.14334/jitv.v28.i3.3148.

Belgian Blue was introduced in Indonesia to increase the biodiversity of livestock genetic resources. Belgian Blue was crossed with Ongole grade to increase the productivity of local cattle. Therefore, this study evaluates reproduction traits, especially the response to superovulation, embryonic development, and quality of Belgian Blue, Ongole grade, and their crosses. Estrous was synchronized with intravaginal progesterone Cue-Mate before artificial insemination (AI). Superovulation was performed with Follicle Stimulating Hormone (FSH) intramuscularly with non-surgical embryo flushing. In addition, embryo quality was assessed microscopically according to the International Embryo Transfer Society (IETS) criteria. The study was performed in a quasi-experimental design, and data were analyzed with an analysis of variance. After superovulation, oocytes/embryos were obtained from all donor breeds. Oocyte and embryo production from Ongole grade and Belgian Blue differed at 11.83±1.91 and 4.86±1.33, respectively, $p<0.05$ (mean ± SEM). In addition, there are differences in recovery rate (89.63% vs. 75.35%) and fertilization rate (77.35% vs. 68.22%) between Ongole grade and Belgian Blue, respectively ($p<0.05$). There is no difference in embryo development quality and proportion of transferable embryos between Ongole grade, Belgian Blue, and their crosses. This study concluded that the cross-bred Belgian Blue x Ongole donor had identical oocyte and embryo production, recovery rate, fertilization rate, and degenerative embryos compared to its purebred.

Keywords: Cattle, Embryo Flushing, *In Vivo*, Pre-implantation, Superovulation

INTRODUCTION

Bovine embryo transfer is essential for reproductive biotechnology to improve female fertility with shorter generation intervals, higher selection intensity, and improved selection accuracy for genetic improvement (Jaton et al. 2016). Approximately 387769 bovine embryos are generated in vivo by superovulation, while more than 1000000 are generated *in vitro* (Viana 2021). In addition, the *in vivo* method results in higher pregnancy success, fewer dead fetuses (Sartori et al. 2016), and high-quality embryos (Marsico et al. 2019).

Variability in individual animal responses to superovulation and the low average number of transferable embryos remain the limiting factors of this technology (Center et al. 2018). Season, physiology, and age differences affect superovulation (Vieira et al. 2014).

Bos taurus and *Bos indicus* exhibit differences in reproductive characteristics. *Bos indicus* has more significant retrieved oocytes due to a greater antral follicle count than Bos taurus (Sartori et al. 2016). They can adapt to heat stress and humidity, rusticity, resistance to endo and ectoparasites (Porto-Neto et al. 2014), poor pasture quality, and a tendency for larger oocytes (de Vasconcelos et al. 2020; de Lacerda et al. 2020; Oliveira et al. 2019; Sales et al. 2015; Guerreiro et al. 2014). However, *Bos taurus* has been subjected to more extensive selection and generally has better productivity, including early maturity (Coffey et al. 2016; Madalena et al. 2015). Therefore, cross-breeding is an alternative to using complementarity and heterosis. Consistent with herd performance improvement, cross-breeding resulted in variation (including reproductive traits) among individuals (Marsico et al. 2021). Jemal et al. (2021) reported that superovulation response was higher in crossbreds (*Bos taurus* x *Bos indicus*).

The Ongole grade is a zebu cattle species widely distributed in the tropical climate of Indonesia. Genetic improvement has been achieved through selection and crossing with other breeds, such as Belgian Blue, a taurine breed recently introduced to improve native livestock diversity. A unique characteristic of this Belgian Blue breed is double muscling due to myostatin mutation. In addition, double-muscled crossbreds are expected to provide better performance production with good adaptability and increase reproduction fertility. Bunning et al. (2019) reported that the heterosis of fertility rate is about 12%. Reproductive traits with low heritability are limited to improvement and can lead to maximum heterosis when crossed (Kirkpatrick 2017).

Donor heritability in embryo production ranges from 0.14-0.19, suggesting that embryo production is influenced by genetics (Jaton et al. 2016). Gomez et al. (2020) found that breeds influence fertility. In addition, Belgian Blue was introduced to improve the performance of local cattle in Indonesia through cross-breeding. A study on the myostatin gene of its crossing with Ongole grade was polymorphic but could not distinguish between normal and double-muscled phenotypes (Jakaria et al. 2021). The crossing with Limousin cattle resulted in better in vitro embryo development and quality than pure Belgian Blue (Residiwati et al., 2020). A previous study showed that embryo production was not different between Belgian Blue, Belgian Blue x Simmental, and Belgian Blue x Holstein (Darlian et al. 2021).

Despite the numerous studies on bovine embryo production, little is known about Belgian Blue embryos derived in vivo from cross-bred animals living in tropical climates. The present study investigated the characteristics of the Belgian Blue x Ongole grade cross-bred and its purebred on reproductive performance as donors in a nucleus breeding program using multiple ovulation embryo transfers (superovulation). The present study aims to determine the effects of subspecies (Bos taurus, Bos indicus, and their crosses) on superovulation response, embryo developmental stage, and quality from Ongole grade, Belgian Blue, and Belgian Blue x Ongole. With this background, the research objectives were (1) to evaluate the superovulation response of Belgian Blue (Bos taurus), Ongole grade (Bos indicus), and their cross (½ *Bos taurus* x ½ *Bos indicus*). (2) Evaluation of the donor breeds' pre-implantation developmental stage and embryo quality.

MATERIALS AND METHODS

Ethical statement

The Ethical Committee, Universitas Padjadjaran, Bandung, West Java, Indonesia, approved the research. The Approval Number is 132/UN6.KEP/EC/2022, registration number 2201050051, dated February 11th, 2022.

Materials

Thirty-three donors consisting of 8 *Bos taurus* (Belgian Blue), 10 *Bos indicus* (Ongole grade), and 15 crosses (½ *Bos taurus* x ½ *Bos indicus*) were used for data collection. From 2017 to 2021, the donor was flushed 22, 35, and 17 times for Belgian Blue, Ongole grade, and crossbreds, respectively. Cross-bred cattle are heifer donors derived from the artificial insemination of Belgian Blue semen with Ongole cows (½ *Bos taurus* and ½ *Bos indicus*). Table 1 shows the donor structure. In addition, 633 oocytes/embryos were obtained from the superovulated donor. This study was conducted at the Livestock Embryo Breeding Centre in Bogor Regency, West Java, Indonesia, at 1240 m above sea level, at 18-22 C and 70-80% relative humidity. All donors were clinically healthy, had a body weight of 475-535 kg, a body condition score of 3.0-3.5, and were not lactating. They were caged in the accessible stall and fed 40-50 kg/day of *Pennisetum purpureum*, 5 kg/day of concentrate, and water ad libitum.

Experimental design

The method used was quasi-experimental with a completely random design. *Bos taurus* (Belgian Blue), *Bos indicus* (Ongole grade), and the cross ½ *Bos taurus*

Table 1. Structure of the number of donors, flushing time, the total number of oocytes/embryos obtained, and physiological condition of cattle

Description	Ongole Grade	Cross-bred	Belgian Blue
Number of donor cattle (n)	10	15	8
Body Condition Score (BCS)	3	3	3.5
Age at superovulation (years)	3-5	3-4	2-4
Number of flushing (times)	35	17	22
Physiological condition of the animal	Not lactating	Not lactating	Not lactating

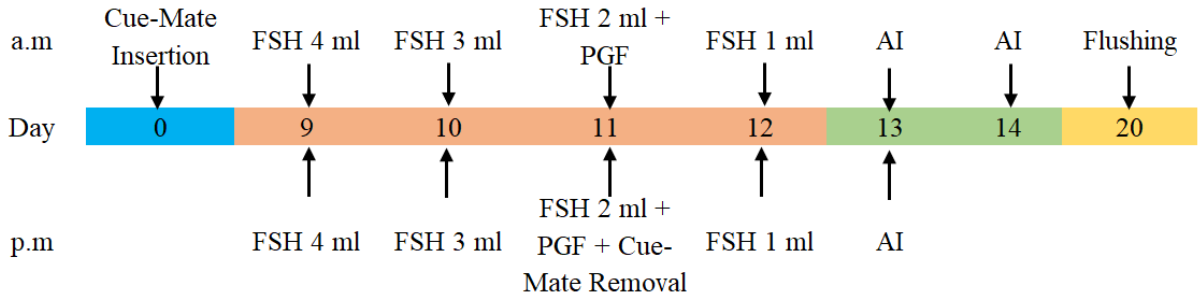

Figure 1. Superovulation protocol based on estrous synchronization

Table 2. Superovulation protocol based on estrous synchronization

Day	Time	Procedures	Description
0	a.m	Cue-Mate insertion	2 pods = 1.56 g progesterone
9	a.m	Treated with 4 ml FSH	FSH Dosage 400 mg/20 ml solvent
	p.m	Treated with 4 ml FSH	Intramuscular
10	a.m	Treated with 3 ml FSH	Intramuscular
	p.m	Treated with 3 ml FSH	Intramuscular
11	a.m	Treated with 2 ml FSH dan PGF2α	Prostaglandin Dosage 2 ml PGF2α
	p.m	Treated with 2 ml FSH, PGF2α, Cue Mate® removal	Intramuscular
12	a.m	Treated with 1 ml FSH	Intramuscular
	p.m	Treated with 1 ml FSH	Intramuscular
13	p.m	First Artificial Insemination (AI)	Interval 8-12 hours
14	a.m	Second AI	Interval 8-12 hours
	p.m	Third AI	Interval 8-12 hours
20	a.m	Identification of corpus luteum	by transrectal palpation
		Flushing and administration of PGF2α	Dosage 2 ml PGF2α
			Ringer Lactate 1,500 ml contains Bovine Serum Albumin and antibiotics

x ½ *Bos indicus* (Belgian Blue x Ongole Grade) were used as treatments. Superovulation refers to the method described by Jodiansyah et al. (2013). Donors were synchronized with 1.56 mg intravaginal progesterone (Cue-Mate®, Bioniche Animal Health Pty. Ltd, Australia) for 11 days, followed by intramuscular injection of 5 mg etiproston in 2 ml prostaglandin (Prostavet C®, Virbac Animal Health, France). In addition, breeds were superovulated with 400 mg NIH-FSH-P1 (Folltrophin V, Bioniche Animal Health Pty.

Ltd, Australia), which was administered for 4 days (a.m:p.m= 4ml:4ml; 3ml:3ml; 2ml:2ml; 1ml:1ml). Donors were inseminated three times (two days after progesterone implant removal in the morning and evening and three days after in the morning) with 0.25 ml of frozen-thawed semen. Belgian Blue semen was used for both Belgian Blue donors (purebred Belgian Blue embryo) and cross-bred (produces ¾ Belgian Blue x ¼ Ongole embryo). In contrast, Ongole grade semen was used for Ongole donors. The non-surgical embryos were flushed on day 7 after the first insemination (on day 20 of the estrous cycle). The estrous synchronization-based superovulation protocol is summarized in Figure 1 and Table 2.

Oocytes/embryo collection and evaluation

Feces were removed from the rectum and perineum before flushing the embryos. The donors were caged, and 4 ml of 2% Lidocaine Hydrochloride was administered for epidural anesthesia between sacrum-coccygeal I or coccygeal I-II to reduce peristalsis and discomfort. They were then assessed by rectal palpation before embryo retrieval, and the corpus luteum was estimated. Embryo flushing was performed 7 days after the first insemination. Donors received uterine lavage with a two-way Folley catheter size 18FR (FHK Fujihira, Jepang). The ringer's lactate was used as the lavage medium containing 1% bovine serum (Sigma-Aldrich, USA) and 100 IU/ml of 0.1% antibiotics penicillin-streptomycin (Sigma-Aldrich, USA). The volume of the embryo collection medium is reduced to approximately 10 ml using a 75 µm diameter filter (Agtech Inc., USA). Then, the results were transferred to a 100x100 mm petri dish (Falcon, USA). The oocytes/embryos collected in the medium are examined and evaluated using a stereomicroscope (Olympus SZ61, Japan) at a magnification of 50-100 times.

Oocytes/embryos were evaluated according to the IETS guidelines (Stringfellow and Givens 2010). Figure 2 shows the pre-implantation development of embryos in the blastocyst (stage 6) from different donor breeds. They were graded according to their quality, with grades 1, 2, 3, and 4 representing Excellent or Good, Fair or Regular, Poor, and Dead or Degenerate, respectively. Transferable (viable) embryos were categorized as grades 1, 2, and 3, while unfertilized and degenerated were defined as untransferable or grade 4 (non-viable). Each stage and grade is briefly described in the IETS manual (Bo and Mapletoft, 2013). Sales et al. (2008) proposed an index for embryo quality, namely the Embryo Quality Index (EQI). This index calculates the oocytes/embryos in each grade with their classification (number of Excellent*1)+(number of Good *2)+(number of Fair*3)+(number of Poor*4)+(number of Degenerate*5)+(number of unfertilized ova*5)/total amount of oocytes/embryos obtained.

Reproductive variables

The reproductive variables measured were the number of oocytes/embryos retrieved, recovery rate, fertilization rate, unfertilized oocytes, degenerated, development stage, grade, and proportion of transferable

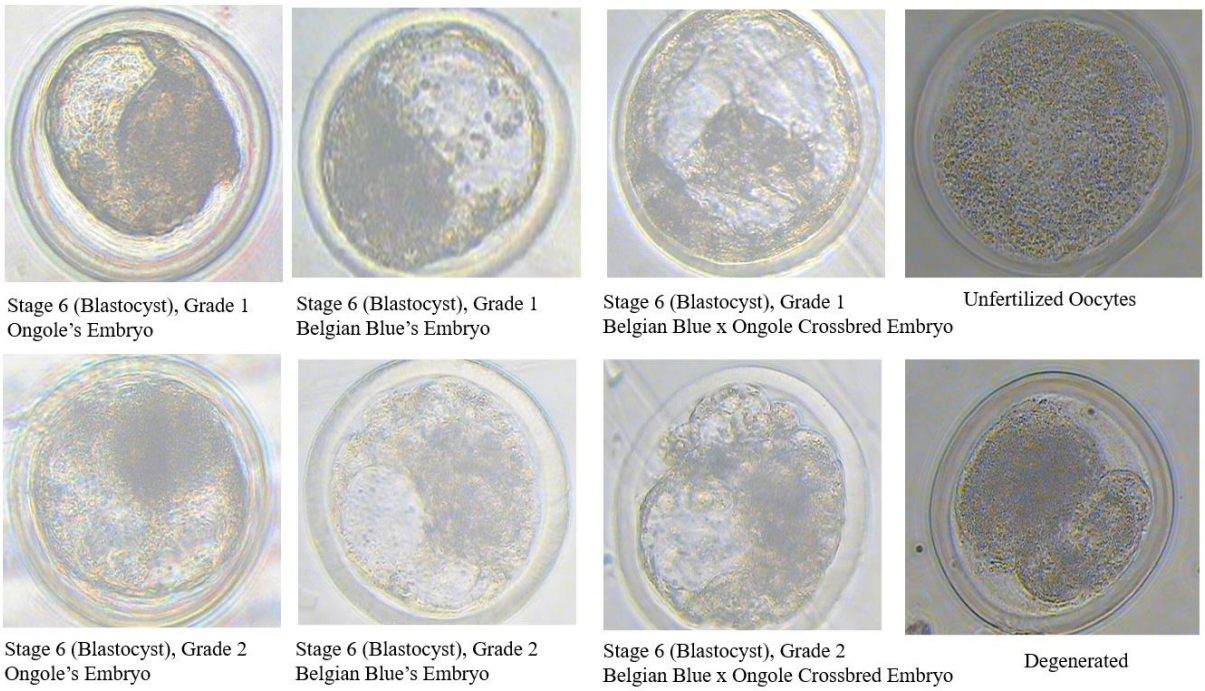

Figure 2. Development of embryos pre-implantation stage

embryos. The following equation was used to calculate the recovery rate, proportion of transferable embryos (viable embryos), and fertilization rate (Sumantri et al. 2011).

$$Recovery\ rate = \frac{Number\ of\ oocyte\ and\ embryo}{Number\ of\ corpus\ luteum} x100\%$$

$$Viable\ embryo = \frac{Grade\ 1,2,3}{Total\ oocyte\ and\ embryo\ recovered} x100\%$$

$$Fertilization\ rate = \frac{Grade\ 1,2,3, Degenerate}{Grade\ 1,2,3\ Unfertilize} x\ 100\%$$

Statistical analysis

The dependent variables were tested for normality using Kolmogorov-Smirnov and Saphiro-Wilk. Analysis was conducted using IBM SPSS Statistics for Windows, Version 26 (IBM Corp., Armonk, NY, USA). The comparison of breed effect on variables observed was analyzed using the Kruskal Wallis test. In addition, the pairwise mean test and significance were set at 95% ($P<0.05$). The following formula shows the model for the Kruskal-Wallis test.

$$H = \frac{12}{N(N+1)} \sum_{i-1}^{k} \frac{R_i^2}{ni} - 3(N+1)$$

where N is the sum of sample sizes in all samples, k is the number of samples, R_i is the sum of ranks in the i^{th} sample, and n_i is the size of the i^{th} sample.

RESULTS AND DISCUSSION

Superovulation response

The recovery rate was significantly different between Ongole grade and Belgian Blue ($P<0.05$) (Figure 3). Ongole grade showed a high recovery rate (89.63%). Oocytes/embryos recovered from Ongole in this study was 11.83±1.91, higher than the result of Imron (2016), which is 9.7±4.9. The main result of the present study showed that Ongole grade cows have the highest recovery rate. Belgian Blue showed a lower recovery rate (75.35%); the average oocytes/ embryos recovered was 4.86±1.33. According to Leroy et al. (2005) and Darlian et al. (2021), superovulated Belgian Blue showed a recovery rate of 87.3% and 75.80%, respectively (Table 3). The difference could be due to climate (subtropic and tropic), physiology (heifers and cows), or the superovulation method. Leroy et al. (2005) used ovulated oocytes as the superovulation response in Belgian Blue raised in subtropic. The superovulation response (calculated as the number of recovered, transferable, degenerated embryos, and unfertilized oocytes), transferable embryos, degenerated embryos, and unfertilized oocytes from Belgian Blue donors raised in the subtropic (numbers and percentage) were 9.2±0.8, 4.2±0.4 (45%), 1.1±0.2 (12%), and 3.9±0.7 (42%), respectively (Leroy et al. 2005).

The cross-bred in this study resulted in a 79.86% recovery rate, which showed no significant difference from its purebred (Table 3). The average oocytes/embryos recovered from cross-bred was 6.53±1.54. Research by Darlian et al. (2021) showed that embryos obtained in vivo from double-muscled crosses between Belgian Blue x Simmental and Belgian Blue x Holstein raised in the tropics did not have different recovery rates with purebred. Garcia et al. (2020) reported that cross-bred produced more oocytes/embryos (11.3±2.0). The optimal heterosis is expected in cross-breeding (Weaber 2015) and is optimized in ½:½ composition (Kirkpatrick 2017). According to Data Retrieval Committee IETS, in 2020 in vivo embryo of bovids was 10 oocytes with 6.2 transferable per flush worldwide (Viana 2021), which indicates low superovulation efficiency (Lonergan et al. 2016). Other factors affecting the superovulation response include the number of follicles in the ovary (Redhead et al. 2018) and the age of the donor (Landry et al. 2016). Donors over 9 years of age produce fewer embryos because fewer follicles to ovulate could respond to exogenous gonadotropins (Landry et al. 2016). The optimal age of the donor is less than 5 years (Jaton et al. 2016); in this study, it was 2-6 years age.

The fertilization rate was different between Ongole grade and Belgian Blue. The Ongole grade showed a high fertilization rate (77.35%) than Belgian Blue (68.22%) and the cross-bred (62.16%). Many unfertilized oocytes (37.84%) affected the fertilization rate in cross-bred cattle. The cross-bred cattle in this study were inseminated with purebred Belgian Blue semen to produce ¾ *Bos taurus* x ¼ *Bos indicus* embryos for upgrading purposes in the nucleus breeding scheme. According to O'Callaghan et al. (2021), sires did not affect fertilization rates, while Marsico et al. (2019) found that significant differences were observed depending on the sire. Research by Sukirman et al. (2019) confirms that the sire breed affects the quality of the semen produced, especially the motility for the fertilization process. In addition, donors in this study were inseminated three times to achieve optimum fertilization. To improve the fertilization rate, in this study, artificial insemination was synchronized in time (Figure 1).

Pre-implantation embryo development stage

As shown in Figure 4, the stage of pre-implantation embryo development (morula to hatched) was not different in all donor breeds. Based on Table 3, the percentage of embryos developed to blastocyst (stage 6) ranged from 13.54% to 26.17% (19.27% cumulative). The percentage of viable embryos that developed (morula to hatched) varied from 49.57% to 54.20%

(52.30% cumulative). In addition, total embryo cleavage (viable and degenerated) ranged from 62.16% to 75.90% (72.19% cumulative). The blastocyst rate of Belgian Blue in this study is higher (19.27%) than reported by Leroy et al. (2005), which is 17.3%.

Pre-implantation embryo development is a complex process involving molecular and structural changes. As development proceeds, control is switched from maternal to embryo-derived transcripts and proteins (Graf et al. 2014). Sudano et al. (2014) reported a higher blastocyst rate in Nelore (a type of Ongole cattle) than in Simmental. Residiwati et al. (2020) note the effect of the Belgian Blue Cross on pre-implantation development. The cross with Limousin resulted in better in vitro embryo development and quality than pure Belgian Blue or Limousin.

Belgian Blue showed fewer degenerated embryos than Ongole grade but higher than the crosses. Higher degenerate embryos in *Bos indicus* could be due to greater lipid droplet composition, which increases sensitivity to oxidative stress and disturbs mitochondrial function (Sudano et al. 2012). *Bos indicus* embryos generated *in vivo* and *in vitro* have a dark appearance, indicating a high lipid composition than taurine (Sudano et al. 2012). In contrast, the pale color of Belgian Blue indicates a lower lipid content (Leroy et al. 2005); this could likely be due to lipid metabolism in double-muscled cattle, but this issue requires further investigation. In this study, the crossing could increase the recovery rate and decreases the percentage of the degenerated embryo, but at the same time, it decreases the fertilization rate. However, those differences were not significant. According to Silva-Santos et al. (2014), a high prevalence of follicular atresia may lead to poor embryo development. In addition, hormones used in superovulation could cause oocyte atresia due to their function inducing follicular development and maturation (Chu et al., 2018).

Table 3. The total number of oocytes/embryos retrieved, recovery rate, embryo development, fertilization rate, embryos quality, and transferable embryos between donors breed (total numbers (percentage, %))

Description	Ongole grade	Cross-bred	Belgian Blue	Total
Total oocytes/embryo recovered (Recovery rate)	415 (89.63)[a]	111 (79.86)[ab]	107 (75.35)[b]	633
Fertilization				
Fertilized	321 (77.35)[a]	69 (62.16)[ab]	73 (68.22)[b]	463 (73.1)
Unfertilized oocytes	94 (22.65)	42 (37.84)	34 (31.78)	170 (26.85)
Development stage				
One cell	94 (22.65)	42 (37.84)	34 (31.78)	170 (26.85)
Morula	13 (3.13)	3 (2.70)	2 (1.87)	18 (2.84)
Early blastocyst	36 (8.67)	3 (2.70)	14 (13.08)	53 (8.37)
Blastocyst	79 (19.04)	15 (13.54)	28 (26.17)	122 (19.27)
Expand blastocyst	73 (17.59)	30 (27.03)	14 (13.08)	117 (18.48)
Hatched	11 (2.65)	4 (3.60)	0 (0)	15 (2.36)
Degenerated	103 (24.82)[a]	14 (12.61)[ab]	15 (14.02)[b]	132 (20.85)
Depeloved and viable (morula to hatched)	212 (51.08)	55 (49.57)	58 (54.20)	325 (52.30)
Cleavage (viable and degenerated)	315 (75.90)	69 (62.16)	73 (68.22)	457 (72.19)
Quality				
Grade 1 (Excellent)	168 (40.48)	39 (35.14)	34 (31.78)	241 (38.07)
Grade 2 (Fair)	49 (11.81)	14 (12.61)	19 (17.76)	82 (12.95)
Grade 3 (Poor)	1 (0.24)	2 (1.80)	5 (4.67)	8 (1.26)
Grade 4 (Discard)	197 (47.47)	56 (50.45)	49 (45.80)	302 (47.71)
Transferable Embryos	218 (52.53)	55 (49.55)	58 (54.20)	331 (52.29)

Different superscripts in the same row showed a significant effect ($P<0.05$)

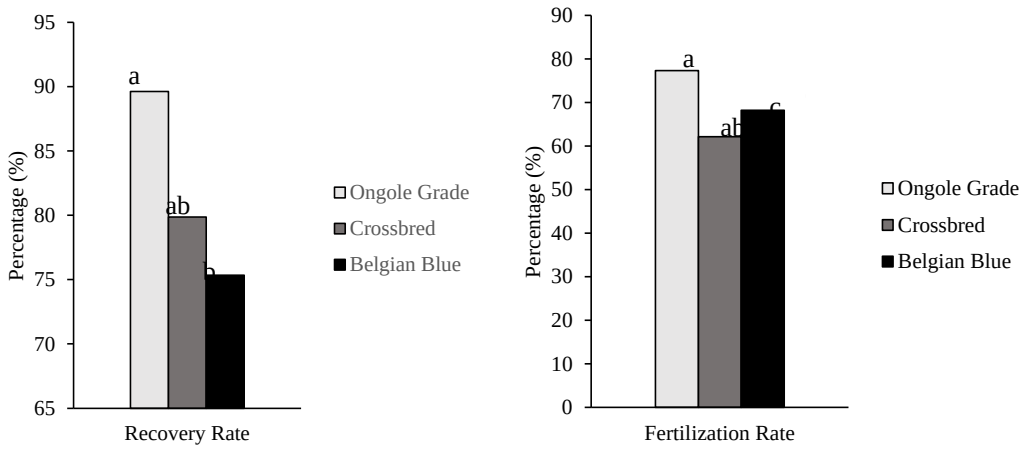

Figure 3. The proportion of recovery rate and fertilization rate from Ongole grade (light bars), cross-bred (gray bars), and Belgian Blue (black bars)

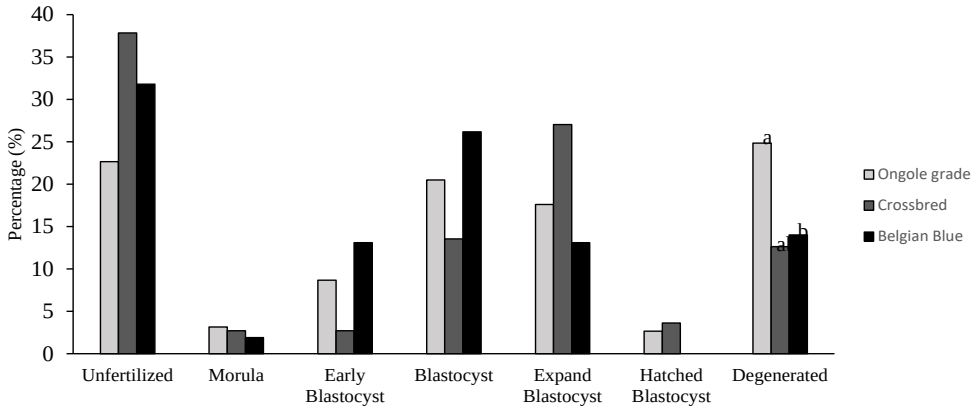

Figure 4. The proportion of development stage from Ongole grade (light bars), cross-bred (gray bars), and Belgian Blue (black bars)

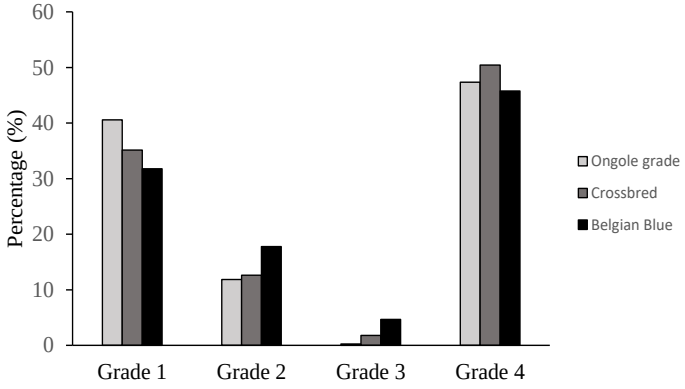

Figure 5. The proportion of embryo quality from Ongole grade (light bars), cross-bred (gray bars), and Belgian Blue (black bars

Other factors related to embryonic development becoming degenerated may correlate with developmental arrest in the early cleavage phase. Embryo molecular competence, associated with development arrest, occurs during the fourth or fourth and fifth cell cycle transition. Maternal RNA or proteins stored in the oocytes are degraded, and the embryonic genome is gradually activated (Graf et al. 2014). Bovine embryonic genome activation occurs between 8 and 16 cells (Graf et al. 2014). Problems during the pre-implantation period, failure of blastocyst hatching, inadequate cell cleavage, and death of the inner cell mass of embryos are all caused by disruption of the endoplasmic reticulum (Luo et al. 2006); this is because the endoplasmic reticulum is the primary mechanism associated with the stress response pathway and disruptions cell function, including cell cleavage (Latham 2015).

Embryo quality

In most mammalian species, especially cattle and humans, the morphological method is the oldest and most widely used in practice to evaluate embryo quality for grading systems. Embryo competence and quality are essential characteristics related to pregnancy rate in embryo transfer and can be explained by molecular, cellular, and morphofunctional factors. The IETS-recommended embryo morphological assessment system specifies several essential variables, including the number and shape of blastomeres, damaged cells, compaction, color, and development stage. This assessment is a standard international reference for embryo trade under the unified and simplified classification system. The Embryo Quality Index (EQI) proposed by Sales et al. (2008) was applied in this study. It yielded identical values ranging from 2.54 to 2.68, with values closer to 1 (one) indicating higher quality embryos (Sales et al. 2008).

In this study, the percentage of embryo quality of each grade was not different for all donor breeds. Based on Table 3, the percentage of the transferable embryos (grades 1, 2, and 3) varied between donors from 49.55% to 54.20% (52.29% cumulative). Belgian Blue produced 54.20% transferable embryos, with 45.80% being grade 4 discarded (unfertilized and degenerated). Naranjo-Chacon et al. (2019) reported that degenerated embryos and unfertilized oocytes from cross-bred with different ages (4-6 years and 8-12 years) showed similarities. A higher proportion of grade 4 was due to a high proportion of unfertilized oocytes (37.84%). Leroy et al. (2005) reported that superovulated Belgian Blue scored 55% Excellent grades, while in this study, only 31.78% were classified as Grade 1 (Excellent).

The high rate of degenerative embryos in superovulated donors may be due to abnormalities in oocyte maturation (Peralta-Torres et al. 2017). Makarevich et al. (2016) reported that the body condition score of donors affects pre-implantation embryo quality. A donor with a BCS of more than 4 produces a poor embryo, as indicated by severely degenerated cells and a fragmented embryo.

The cytoplasmic lipid content of the embryo is another aspect that affects embryo quality. Due to the low lipid content, the Belgian Blue embryo has a transparent appearance (Leroy et al. 2005). According to Ordonez-Leon et al. (2014), the cytoplasmic lipid of *Bos taurus* embryos was higher than *Bos indicus*. Nelore embryos had higher phospholipids (mainly phosphatidylcholine), which gave them a darker appearance than Simmental embryos (Visintin et al. 2002). Since *Bos taurus* was naturally selected under cold temperatures, this could affect oocyte and lipid composition in the embryo (Sudano et al. 2012). Marsico et al. (2021) demonstrated that triacylglycerol predominates in *Bos taurus* embryos. Triacylglycerol forms lipid droplets in the cytoplasm and serves as an energy source and cell signaling pathway for mammalian embryonic development.

CONCLUSION

This study concluded that: (1). Oocytes/embryos were successfully obtained from all donor cattle breeds with the production of oocytes/embryos from Ongole grade higher than Belgian Blue. (2). The cross-bred donor had identical oocyte and embryo production and quality compared to the purebred.

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Meat Quality Characteristics of IPB-D1 Chicken and the Final Stock from Different Locations

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ABSTRAK

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Peningkatan permintaan protein hewani mendorong pengembangan inovasi jenis atau bangsa baru ternak. Ayam IPB-D1 merupakan ayam lokal hasil seleksi yang menghasilkan peningkatan produktivitasnya. Pemeliharaan ayam IPB D1 dilakukan di Kabupaten Sukabumi dan Kabupaten Bekasi. Pengujian kualitas fisik, kimia dan mikrobiologi daging ayam IPB-D1 dilakukan di Laboratorium IPTP Terpadu dan Laboratorium Mikrobiologi Hasil Ternak, sementara pengujian organoleptik dilakukan di Ruang Organoleptik, Fakultas Peternakan, IPB University. Penelitian dilaksanakan pada Februari sampai Juni 2022. Ruang lingkup penelitian ini mencakup pemeliharaan ayam yang dilaksanakan pada dua lokasi berbeda dan pengambilan sampel yang bertujuan untuk pengujian kualitas fisik, kimia, mikrobiologi dan organoleptik. Ayam yang di ujikan adalah ayam IPB-D1 Sukabumi, ayam IPB-D1 Bekasi, ayam IPB-D1 *final stock* Sukabumi, ayam IPB-D1 *final stock* Bekasi dan ayam Kampung, Sentul, dan broiler yang terdiri dari 15 ekor masing-masing. Pada tiap lokasi tersebut pengambilan sampel dilakukan sebanyak 10 kali sebagai ulangan. Teknik pengambilan sampel yang digunakan adalah simple random sampling. Hasil penelitian menunjukkan kualitas daging ayam IPB-D1 dan final stocknya menunjukkan hasil tidak berbeda nyata ($P>0.05$) pada aspek kualitas fisik daging yang berupa pH dan Daya Mengikat Air (DMA) tetapi memiliki pengaruh yang signifikan ($P<0.05$) pada parameter susut masak dan keempukan apabila dibandingkan dengan ayam Kampung, Sentul dan broiler. Sementara itu, kualitas kimia daging ayam IPB-D1 dan final stocknya menunjukkan hasil yang berbeda signifikan ($P<0.05$) pada kandungan lemak daging. Adapun untuk parameter kadar abu, protein, mineral dan kolesterol menunjukkan hasil yang tidak berbeda signifikan ($P>0.05$). Analisis kualitas mikrobiologi berupa total koloni bakteri (total plate count) daging ayam IPB-D1 dan Final Stocknya yakni sebesar 105 CFU/g. Hasil tersebut masih dalam Batas Maksimum Cemaran Mikroba yang ditetapkan oleh Badan Standarisasi Nasional Indonesia tahun 2009. Berdasarkan hasil penelitian ini, perlu dilakukan perbaikan cara penanganan, pengemasan yang higienis hingga sanitasi agar kualitas daging ayam dapat ditingkatkan, dan cemaran mikroba dapat diminimalkan.

Kata Kunci: karakteristik, daging ayam, final stock, IPB-D1, mikrobiologi, organoleptik

ABSTRACT

Adelta KB, Arief II, Sumantri C, Wulandari Z. 2023. Meat quality characteristics of IPB-D1 chicken and the final stock from different locations. JITV 28(3):197-207. DOI:http://dx.doi.org/10.14334/jitv.v28.i3.3121.

The increasing demand for animal protein encourages innovations development of new livestock types or breeds. IPB-D1 chicken is an improved local chicken for their productivity. Its rearing was carried out in Sukabumi and Bekasi Regency. Physical, chemical, and microbiological quality testing of IPB-D1 chicken meat was carried out at the Integrated IPTP Laboratory and the Microbiology Laboratory of Livestock Products, and organoleptic testing was carried out in the Organoleptic Room, both of the Faculty of Animal Husbandry, IPB University. The research was carried out from February to June 2022. The scope of this research includes chicken rearing at two locations and sampling that aims to test the physical, chemical, microbiological, and organoleptic quality. The chickens tested were the IPB-D1 Sukabumi chicken, IPB-D1 Bekasi chicken, IPB-D1 final stock Sukabumi chicken, IPB-D1 Bekasi final stock chicken, and Kampong, Sentul, and broiler chickens, which consist of 15 chickens each. At each location, sampling was carried out 10 times as a replication. The sampling technique used is simple random sampling. The results showed that the quality of IPB-D1 chicken meat and its Final Stock did not have a significant difference ($P>0.05$) in physical quality aspects such as pH and water holding capacity (WHC) but had a significant difference ($P<0.05$) in cooking loss and tenderness than Kampong, Sentul, and broilers. Meanwhile, the chemical quality of IPB-D1 broilers and their final stock showed significantly different results ($P<0.05$) in the fat content of the meat. As for the parameters of ash content, protein, minerals, and cholesterol showed no significant difference ($P>0.05$). Microbiological quality analysis in the form of total bacterial colonies (total plate count) of IPB-D1 broilers and its Final Stock was 105 CFU/g. These results are still within the Maximum Microbial Contamination Limits set by the Indonesian National Standardization Agency in 2009. Based on the results

of this study, it is necessary to improve handling methods, hygienic packaging, and sanitation so that the quality of broilers can be improved and microbial contamination can be minimized.

Key Words: Characteristics, Chicken Meat, Final Stock, IPB-D1, Microbiology, Organoleptic

INTRODUCTION

The increasing public demand for animal protein encourages the birth of various innovations related to the development of new breeds and types of livestock that can grow with high productivity and a relatively fast time. The consumption of chicken meat in Indonesia increases every year. The average amount of chicken meat consumed per capita daily in Indonesia in 2021 is 0.02 kg (BPS 2022). One of the livestock commodities that are in great demand by the community is local chicken. Local chicken has a distinctive taste, but its productivity is lower than broilers (Mahmud et al. 2017). Various efforts continue to be made to increase the productivity of local chickens, namely by conducting various research and development. One of the results is IPB-D1 Chicken.

The chickens of IPB-D1 have been officially announced by the Indonesian Ministry of Agriculture based on Decree No.693/KPTS/PK.230/M/9/2019 as a new family of composite local chickens. Currently, various studies through the application of molecular genetics are widely used by experts to increase the productivity and quality of local Indonesian chickens. Based on Sumantri & Darwati (2017), the chickens of IPB-D1 are the result of crosses between Pelung chicken, Sentul chicken, Kampong chicken, and broilers. The superiority of the IPB-D1 chicken is because, genetically, it is a chicken composite with varying gene diversity from each parent Sumantri & Darwati (2017). The slaughter weight of IPB-D1 chickens reached 1.2−1.7 kg at the age of 12 weeks Sumantri and Darwati (2017). Sumantri & Darwati (2017) reported that IPB-D1 chickens had similar body weight performance characteristics at the age of 10-12 weeks of slaughter, body weight at 12 weeks of age in hens ranged from 1.04±119.24 grams, and as for roosters about 1.18±203.4 gr. Sumantri & Darwati (2017) added that IPB-D1 chickens are adaptable and can develop well despite being in a Tetelo endemic area. IPB-D1 chickens can be further developed to produce higher productivity with faster growth.

IPB-D1 chickens have been developed in various rearing sites with different conditions and rearing management; this allows for differences in the quality of the resulting chicken meat. The chickens of IPB-D1 have the character of having more remarkable body weight growth than Kampong chickens and are more resistant to disease than purebred chickens (Susanti et al. 2020). Many things, including public perception, influence the success of innovation in being accepted and developed in society. Perception can play one of the important roles in determining an innovation, in this case, the IPB-D1 chicken, so that the wider community can accept it. It can continue to be developed to the industrial stage. One of the things that can be done to shape public perception is by conducting further research on IPB-D1 chickens, especially regarding the physical and chemical quality of meat, microbiological, and consumer acceptance. The meat quality in question includes physical, chemical, and biological qualities as well as organoleptic. Therefore, further research is needed to determine the physical, chemical, and organoleptic qualities of IPB-D1 chickens from various locations and rearing management systems.

MATERIALS AND METHODS

Location

The maintenance of IPB-D1 chickens is carried out by partners of the Faculty of Animal Husbandry of IPB intensively with unique methods of rearing located in Sukabumi Regency (CV Sinar Harapan Farm) and Bekasi Regency (CV Citra Lestari Farm). Tests for the physical and chemical quality of meat and microbiology are carried out at the Integrated Laboratory of the Faculty of Animal Husbandry IPB, Laboratory of Animal Husbandry Microbiology, Faculty of Animal Husbandry IPB, and Inter-University Center Laboratory of IPB. Organoleptic testing was conducted in the Organoleptic Laboratory of the Faculty of Animal Science IPB.

Materials

The tools used for physical analysis are a pH meter's meat, aw meter, chromameter, biuret, measuring flask, pipette, beaker, and Erlenmeyer flask. This study also used a freezer (-18°C), Petri dishes, hot plate stirrer, test tubes, Erlenmeyer tubes, digital scales, measuring cups, volumetric pipettes, micropipettes, Pasteur pipettes, Bunsen heaters, aluminum foil, plastic wrap, tips, sealer, caliper, incubator, refrigerator, autoclave, oven, vortex, sprayer, paper disc, and burette. This study used chicken breast, which was taken by sampling from 3 locations. The final stock chicken comes from Bekasi. The chickens of IPB-D1 came from Sukabumi and Bekasi. The Sentul, Kampong, and Broiler chicken was purchased from Sukabumi and Bogor, each with 15 chickens for comparison study. At each location, sampling was carried out 9 times as replication. The sampling taken using the technique is simple random sampling. Samples were taken in the morning to prevent

increased contamination in the open area. Furthermore, the meat samples were put into sterile plastic, labeled per each sample with a different location, and put in a coolbox, then brought to the laboratory for further testing.

Chicken meat sample preparation and effect of maintenance management pattern

The chicken meat was obtained from the slaughter of IPB-D1 chickens, and the final stock was taken from the chest and thighs. The final stock chickens came from Bekasi (CV Citra Lestari Farm) and Sukabumi (CV Sinar Harapan Farm). The chickens of IPB-D1 came from Bekasi (CV Citra Lestari Farm) and Sukabumi (CV Sinar Harapan Farm). For the analysis of physical properties, 45 samples of chicken breast were taken with fifteen chicken meat from IPB-D1 Bekasi (CV Citra Lestari Farm), fifteen chicken meat from IPB-D1 Sukabumi (CV Sinar Harapan Farm), fifteen chicken meat from IPB-D1 Bekasi. The final stock came from Bekasi (CV Citra Lestari Farm), fifteen final stock chickens from Sukabumi (CV Sinar Harapan Farm), five free-range chickens, five Sentul chickens, and five broiler chickens. The chicken meat used as material for analyzing physical properties, chemical properties, and total microbes was carried out by a separation process between the meat and bone parts (deboning). For organoleptic testing, use the chicken breast as much as 200 grams of each chicken in small pieces. In addition to taking samples, IPB-D1 chickens and their final stock were checked to see how they correlated with the chicken-rearing management system at each location.

Physical quality analysis of meat samples

Meat pH

Measurement of meat pH is carried out with a unique pH meter for meat. The pH meter was previously calibrated at pH 4 and 7. The electrodes were rinsed with aqua dest and dried. Then 10 grams of chicken meat are mashed using a blender by adding 100 ml of water until homogeneous for one minute; the blender results are poured into a measuring cup. The electrode will go into the sample, and the pH value will appear on the pH meter. If the measurement of the pH value is carried out on different meat samples, the tip of the pH meter should be washed first using distilled water and then dried with tissue paper. The pH value listed on the pH meter display is read and recorded.

Water Holding Capacity (WHC)

Measurement of the water holding capacity value using a carper press and a planimeter. The initial stage of measuring the water holding capacity is a 0.3 g meat sample, which was accurately weighed using a Sartorius scale, placed between Whatman 41 filter paper, and then pressured using a carper press (35 $kgcm^{-2}$) for 5 minutes. Two circular areas show the meat under pressure (Inner Circumference = LD) and the water from the meat sample (Outer Circumference = LL or wet area). LD and LL on Whatman filter paper number 41 are marked with a pen. The amount of free water that comes out is measured using a planimeter. The formula calculates the value obtained from the measurement results, according to Soeparno (2016).

Meat tenderness

Texture testing was done using a texture analyzer (TA) to test cooked chicken meat's hardness, springiness, cohesiveness, fractubility, gumminess, and chewiness. The probe used in this analysis is cylindrical. A sample with a thickness of 1 cm is placed on top of the testing sample; then, the load cell will move the probe down to press the sample and back up. The texture analyzer's working principle is the product's durability by the compressive force of the tool or the ability to return the pressed food material to its initial condition after the pressure load is removed (Soeparno 2016).

Cooking loss

The cooking loss can be calculated by weighing the sample before boiling as the initial weight. The sample was pierced with a bimetallic thermometer and then boiled in boiling water until the internal temperature of the meat reached 71-81^{o}C. The sample was then removed and weighed. Cooking loss is calculated based on the formula according to Bouton (1971).

Chemical quality analysis of meat samples

Proximate analysis

Chemical quality analysis was observed by proximate test on parameters of moisture, ash, fat, and protein content—analysis using a food scanner NIRS (Near Infrared Reflectance Spectroscopy). Samples of 30 grams were chopped, then checked using a special petri dish. Sample examination was carried out in triplicate.

Mineral content analysis

The mineral content analysis was done following Fahruzaky et al. (2020) methods. Testing for mineral content uses X-Ray Fluorescence (XRF) which produces quantitative data on specific mineral levels. Before measuring the mineral content in the sample,

measurement, and energy calibration are first carried out. Energy calibration aims to keep the elements contained in a material at its energy level, while measurement calibration aims to determine measurement deviations from the tool. The sample is inserted into the XRF device. The working principle of this tool is irradiating X-rays into the sample so that the photoelectric effect is obtained and then displays the mineral content.

Cholesterol level analysis

A total of 50 mg of chicken meat extract, each part of the thigh and breast in a composite, was put in a 25 mL flask and dissolved with chloroform to the mark. About 1 mL of sample solution was added to 2 mL of Liebermann-Burchard reagent in a 5 mL volumetric flask, then filled to the mark with chloroform. Each mixture was incubated for 5 minutes. The absorbance was measured at the maximum wavelength. The solution was made three times. Cholesterol levels were calculated by the formula following the research of Sahriawati et al. (2019).

Chicken meat microbiological quality

Total Plate Count (TPC) analysis

TPC calculation by pour plate method. A total of 25 g of mashed chicken meat samples were put into 225 ml of sterile 0.1% Peptone Water (BPW) media and obtained a dilution of 10^{-1} (P1). A total of 1 ml of suspension from P1 was transferred with a sterile pipette into 9 ml of sterile 0.1% BPW medium until a dilution of 10^{-2} (P2) was obtained. Do the same way until the dilution is obtained up to 10^{-7} (P7). Each 1 ml of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions was taken to be put into a sterile petri dish and carried out in duplicate. Furthermore, prepared previously, 10-15 ml of agar plate count (Oxoid CM 0325) is poured into a petri dish. Smooth it over the entire surface of the cup. The next step is to incubate at 37-38 ^{0}C in an inverted dish for 24-48 hours. Colony counting was done using a colony counter based on the Standard Plate Count (SPC) provisions.

Organoleptic test

A hedonic quality test was conducted to assess the acceptability and quality of food products by rating test method using 5-value intervals. The variables assessed included color, the intensity of chicken breast meat aroma, and the intensity of mucus. The organoleptic quality testing of each treatment combination was carried out simultaneously. The test was conducted on 40 semi-trained panelists. Based on this hedonic organoleptic quality test, it is known that the panelists still accept the best treatment and the suitability of the meat for consumption**.**

Data analysis

The design used was a *Completely Randomized Design* (CRD). Data on meat's physical and chemical quality and microbiology were analyzed using the t-student test (Mattjik and Sumertajaya, 2013). The *Duncan Multiple Range Test* (DMRT) was performed if there was a difference between the three locations with a 95% confidence interval. Organoleptic data were tested using a 95% confidence interval. Data that does not meet the statistical rules are described descriptively. The treatment level with a significant or very significant effect is tested for significant differences in the mean value using the Mann-Whitney test.

RESULTS AND DISCUSSION

Physical quality

Factors of texture, color, taste, tenderness, smell, and juiciness can influence consumers' assessment of meat quality before buying meat. Poultry production management is reflected mainly in the meat's juiciness, tenderness, and taste (Mir et al. 2017). Analysis of the physical properties of IPB-D1 chicken meat using chicken breast. The chickens of IPB-D1 in this study were differentiated based on different rearing and management locations. The results of testing the physical properties of IPB-D1 chicken meat can be seen in Table 1.

pH value of meat chicken

Physical quality analysis in the form of the pH value of IPB-D1 meat reared at different locations and free-range chicken, Sentul, and broiler as comparison are shown in Table 1. The location of IPB-D1 chicken rearing is different, and free-range, Sentul, and broiler chickens, for comparison, had no significant effect on the meat pH. The results of this study showed that the pH of meat at various rearing locations was in the range of 5.90 to 6.04; this indicates that the difference in the location of rearing chickens IPB-D1 did not cause a significant change in the pH value of the meat when compared to kampong, Sentul, and broiler chickens.

Table 1. Analysis of the physical quality of IPB-D1 meat reared at different locations and Kampong chicken, Sentul, and broiler meat for comparison

Parameter	Rearing Location						
	IPB D1 Sukabumi Chicken	IPB D1 Bekasi Chicken	D1 Sukabumi Final Stock Chicken	D1 Bekasi Final Stock Chicken	Kampong Chicken	Sentul Chicken	Broiler Chicken
pH	5.90±0.25	5.93±0.19	6.01±0.30	6.04±0.16	5.93±0.13	5.98±0.15	6.00±0.07
Water Holding Capacity (% mg H2O)	31.48±3.27	31.28±3.78	30.19±1.79	30.72±3.00	31.28±0.98	30.52±1.97	30.29±2.42
Cooking Loss (%)	12.94±4.1^{ab}	15.35±3.1^{a}	14.48±2.9^{a}	14.20±4.5^{ab}	16.37±0.8 ^{ab}	17.04±0.2^{ab}	8.37±0.7^{b}
Tenderness (gr cm $^{-1}$)	2.95±0.8^{a}	2.77±0.^{ab}	2.93±0.7^{b}	2.74±0.8^{b}	3.13±0.7^{ab}	2.93±0.7^{ab}	2.76±0.2^{ab}

*Different superscripts in the same row showed significantly different effects of the 5% Tukey test (P<0.05)

The average pH value in this study was still within the normal range. Soeparno (2016) reported that the pH of fresh chicken meat ranged from 5.3-6.5 under normal conditions after slaughter. The optimal pH value of broiler chicken meat without treatment is 5.78 (Benamirouche et al. 2020). The average pH value of IPB-D1 Sukabumi chickens was the lowest (5.90) when compared to other locations, followed by IPB-D1 Bekasi chickens (5.93), Kampung chickens (5.93), Sentul chickens (5.98), Broiler Chicken (6.00), Sukabumi Final Stock Chicken (6.01) and the highest meat pH value (6.04) was D-1 Bekasi final stock chicken. This study showed that the pH value of chicken meat was higher than in the study of Afrianti et al. (2013), with broiler chicken meat without any treatment having an average pH of 6.79 in a shelf life of 6-12 hours.

pH value is one of the important indicators for assessing the physical quality of meat. It can determine the presence of microbes in meat, so it dramatically determines the level of quality and durability (Hajrawati et al. 2016). Biochemical changes after slaughter cause the conversion of muscle to meat which determines the quality of meat at the end. Carcass temperature after slaughter has a physicochemical effect on muscle associated with postmortem glycolysis, temperature, and pH (Mir et al. 2017). Changes in the pH of meat after slaughter are influenced by the availability of lactic acid in the muscles; the availability of lactic acid is influenced by glycogen content, and livestock handling influences glycogen content before slaughter. The pH value directly influences meat quality, such as tenderness, water-holding capacity, color, juiciness, and shelf life. Broiler breast meat with a high pH has a higher water-holding capacity than meat with a low pH (Mir et al. 2017), presumably because the chickens used to have different body weights. Glycogen content was higher in chickens with higher body weight, resulting in higher levels of rigor mortis. Color identification is an easy way to determine the pH of meat. If the meat is very dark, then the pH of the meat is high, and if it is very light, it has a low pH.

Water Holding Capacity (WHC)

The water-holding capacity of IPB-D1 chickens from different rearing locations and free-range, Sentul, and broiler chickens for comparison are shown in Table 1. The different locations of IPB-D1 chicken rearing and free-range, Sentul, and broiler chickens as comparisons had no effect. Significant on the water-holding capacity of meat, this indicates that the location of rearing in IPB-D1 chickens did not cause changes in the water-holding capacity of the meat, and when compared to native chickens, Sentul chickens, and broiler chickens, there was no significant difference. The water-holding capacity of the meat from this study at various rearing locations was the lowest shown by Final Stock D-1 Sukabumi chicken (30.19%) to the highest 31.48% (IPB-D1 Sukabumi chicken). Meanwhile, for other locations, the water holding capacity was 31.28% (chicken IPB-D1 Bekasi); 31.28% (village chicken); 30.72% (D-1 Bekasi Final Stock chicken); 30.52% (Sentul chicken), and 30.29% (broiler chicken).

The findings of Mahmud et al. (2017) reported the water-holding capacity of broiler chicken meat with different cage densities of about 33.65-34.28%. This study's water-holding capacity (WHC) value is around 30,19-31.48%, which is lower than the findings of Mahmud et al. 2017. The low pH of the meat can reduce the water-holding capacity due to the open structure of the meat. Likewise, high-pH meat can increase the water-holding capacity because the meat structure is closed.

Cooking Loss

The cooking loss of IPB-D1 chicken from different rearing locations and native chicken, Sentul, and broiler chickens as comparisons are shown in Table 1. The different locations of IPB-D1 chicken rearing and native, Sentul, and broiler chickens as comparisons had a significant effect. ($P<0.05$) on the cooking loss of meat. The cooking lossof meat from this study was in the range of the lowest 8.37% (broiler chicken) to the highest 17.04% (Sentul chicken).

Meanwhile, IPB-D1 Bekasi chickens amounted to 15.35%, followed by IPB-D1 Sukabumi chickens at 12.94%, Final Stock D-1 Sukabumi chickens (14.48%), and Bekasi D-1 Final Stock chickens (14.20%). The results are pretty varied, but according to Soeparno (2016), cooking loss generally varies between 1.% - 54.5%, with a standard range of 15% to 40%. The findings of this study are lower when compared to Mahmud et al. (2017), with a cooking loss value of 34-36%. The cooking loss value of chicken meat in this study was lower than the findings of Mahmud et al. 2017. Moreover, better when compared to the results of Khaerunnisa et al. (2016). Mir et al. (2017) reported that changes in carcass quality associated with unsaturated fatty acids could tear the skin during picking and increase cooking loss. Poultry subjected to heat stress before slaughter generally has a higher body temperature, resulting in a rapid decrease in pH and the development of muscle stiffness. Such pre-cutting conditions usually produce pale, soft, and exudative meat, resulting in lower yields, increased cooking losses, and reduced juices.

Tenderness

Tenderness of IPB-D1 chicken meat from different rearing locations and free-range, Sentul, and broiler chicken meat as comparisons shown in Table 1. Different locations of IPB-D1 chicken rearing as well as free-range chicken, Sentul chicken, and chicken broiler, as a comparison, had a significant effect ($P<0.05$) on the tenderness of the meat. The tenderness of the meat significantly increased in Final Stock D-1 Bekasi chicken (2.74 gr/cm), followed by IPB-D1 Bekasi chicken (2.77 gr/cm). Meanwhile, the lowest meat tenderness was free-range chicken (3.13 g/cm). The chickens of IPB-D1 chicken breasts were reared in different locations, and when compared to native chickens, Sentul chickens and broiler chickens were included in the very tender category based on the grouping of tenderness. The findings of Mahmud et al. (2017) reported that the tenderness value of broiler village crosses was 1.17-1.58 kg/cm^3.

The study results showed a better tenderness value when compared to the findings of Ariyanti et al. 2019. The cooking process can affect the tenderness of the meat, which causes the myofibril proteins to coagulate and denature. Physically, myofibril proteins react due to heating so that hardening occurs, affecting the meat's tenderness (Mahmud et al. 2017). The chickens of IPB-D1, which are the result of crosses between Pelung chickens, Sentul chickens, native chickens, and broilers, have become a new genetic variation in the poultry sector; this is supported by Mir et al. (2017) reported that differences in quality could be due to genetic variation among birds. Chicken meat quality can be improved by genetic selection. Meat tenderness can be affected by connective tissue and myofibrillar proteins along with heat, environmental stress, poultry, and developmental rigidity.

Chemical quality

Chemical quality analysis of IPB-D1 chicken meat to determine the proximate content of the parameters of water content, ash, fat and protein, mineral content, and cholesterol content. The average and standard deviation results from testing the chemical properties of IPB-D1 chicken meat are shown in Table 2. The high nutritional content of meat makes it a product the body needs. The chemical composition of meat differs in number depending on the species, genetics, age, carcass, storage, sex, nutrition, and handling process of livestock (Liur 2020). The chemical quality of meat is influenced by water, fat, and protein content (Prasetyo et al., 2013). Water, protein, and fat content affect the chemical quality of meat (Prasetyo et al., 2013).

Proximate

The different locations of rearing IPB-D1 chickens as well as native, Sentul, and broiler chickens as a comparison, did not have a significant effect ($P>0.05$) on the proximate results in the form of ash and protein content. However, there was a difference in fat yield. Quantitatively, the ash content of chicken meat at various rearing locations was the lowest at 0.81% (D-1 Bekasi Final Stock chicken) to the highest at 1.22% (Sentul chicken). Meanwhile, the ash content of other locations, such as IPB-D1 Sukabumi chickens, was (1.13%), and IPB-D1 Bekasi chickens (0.85%). These results align with Mahmud et al. (2017) on broiler crossbreed chicken meat by 1.08-1.15%. The research findings of Liur (2020) are 0.74% of broiler chicken meat in traditional markets. According to Tamzil (2014), fresh chicken meat contains an ash content of 1.14%. Ash content is a determining factor for nutritional content related to mineral content in chicken meat. Ash content can increase with the increasing age of livestock. Qurniawan et al. (2016), the ash content increased with the increasing age of broiler chickens.

Table 2. Analysis of the chemical properties of IPB-D1 meat reared at different locations and chicken, Sentul, and broiler meat for comparison using a composite method

Parameter		Rearing Location						
		IPB-D1 Sukabumi Chicken	IPB-D1 Bekasi Chicken	D1 Sukabumi Final Stock Chicken	D1 Bekasi Final Stock Chicken	Kampung Chicken	Sentul Chicken	Broiler Chicken
Proximate	Ash (%)	1.13±0.20	0.85±0.04	1.09±0.14	0.81±0.13	1.08	1.22	0.93
	Fat (%)	1.37±0.4^{ab}	0.36±0.1^{b}	3.06±1.5^{ab}	1.18±0.5^{b}	2.06^{ab}	0.33^{b}	5.43^{a}
	Protein s (%)	20.90±1.41	21.30±0.44	18.62±0.90	20.69±0.42	20.59	21.45	19.53
Mineral	Fe (ppm)	8.35±1.89	11.23±4.04	14.56±0.02	7.48±5.16	3.26	7.69	14.92
	Zn (ppm)	12.27±6.21	23.74±2.24	21.75±4.38	11.01±7.53	4.77	11.21	20.09
Cholesterol	(mg/100g)	78.47	69.49	47.53	60.72	116	164.80	110

Different superscripts in the same row showed significantly different effects of the 5% Tukey test (P<0.05)

The amount of ash content is also related to the level of livestock consumption. The higher the level of consumption, the higher the ash content. The amount of ash content varies depending on sex, species, and age.

Analysis of the variety of meat fat content showed that the location of IPB-D1 chicken rearing affected the fat content of the meat(P>0.05). The lowest fat content was shown in Sentul chicken (0.33%), followed by IPB-D1 Bekasi chicken (0.36%) and IPB-D1 Sukabumi chicken (1.37%). The highest fat content in broiler chickens is 5.43%. Hartono et al. (2013) explained that fat content was negatively correlated with meat protein content. The lower the fat content of the meat, the higher the protein content of the meat, and vice versa. The nutrient digestion and metabolism of livestock influence the chemical quality of meat. In addition, the older the livestock, the fat content will also increase. Fat content is also related to livestock weight; the greater the weight of the chicken, the greater the fat content.

The protein content of meat showed that the location of rearing IPB-D1 chickens and, when compared with sentul chickens, free-range chickens, and broilers, did not affect the protein content of the meat (P>0.05). The lowest protein content of meat was shown by Final Stock D-1 Sukabumi chicken (18.62%), followed by broiler chicken (19.53%). The protein content of IPB-D1, Sukabumi, and Bekasi chicken meat was high at 20.90% and 21.30%, respectively. The results of the meat protein content found by the Liur study (2020) were 21.96%. Research Chepkemoi et al. (2017) reported an indigenous chicken protein content of 15.1%. The protein content in the study was still within that range, so it was considered normal. According to Liu et al. (2015), the amount of feed consumed by livestock will affect the protein content of meat. Rotiah et al. (2019) added that high protein content is also associated with animal weight. Chicken body weight is related to protein consumption which determines the protein deposition of chicken meat. The main components of protein are amino acids that form long chains consisting of essential and non-essential amino acids.

Mineral

Chemical quality analysis of IPB-D1 chicken meat protein content from different rearing locations and free native chicken, Sentul, and broiler as comparisons are shown in Table 2. The location of different IPB-D1 chicken rearing and free native chicken, Sentul, and broiler as comparisons had no significant effect (P>0.05) on the mineral content of Fe and Zn. The Fe and Zn mineral content results in this study were quite varied. Quantitatively, the highest Fe mineral content (14.92 ppm) in broilers and the lowest (3.26 ppm) in native chickens. The highest Zn mineral content (23.74 ppm) in IPB D1 Bekasi chickens and the lowest (4.77 ppm) in native chickens.

According to Benamirouche et al. (2020), the mineral Zn is a cofactor of the antioxidant enzyme superoxide dismutase (SOD) in fighting free radicals and is needed for acid and alkaline balance. Zinc is needed by the body of livestock in small quantities, but its presence cannot be stored in the body, so zinc intake from the feed is needed because zinc cannot be converted from other nutrients (Swain et al. 2016; Chepkemoi et al. 2017). Increasing the concentration of zinc and vitamin E in the feed can reduce the concentration of malondialdehyde

(MDA) under stress conditions. Livestock can experience oxidative stress due to high environmental temperatures. Adding zinc minerals to the diet can prevent the occurrence of lipid peroxides and increase the immune system in tissues (Kakhki et al. 2016).

Cholesterol level

Table 2 shows the results of the analysis of chemical properties in the form of cholesterol levels of IPB-D1 chicken meat from different rearing locations as well as free-range chicken, Sentul, and broiler as comparisons. The cholesterol content of meat is quite varied, with a range of 47.53 mg/100g (final stock chicken). D-1 Sukabumi) followed by 60.72 mg/100g (D-1 Bekasi final stock chicken). The cholesterol levels of IPB-D1 Sukabumi and Bekasi chickens were 78.47 mg/100g and 69.49 mg/100g, respectively. This result is higher than Mahmud et al. (2017), 18-20 mg/100 g. The data from this study showed that the cholesterol of IPB D1 Sukabumi and Bekasi chickens was lower than that of native chickens and broilers. The lower cholesterol value in broiler chickens is thought to be due to heterosis. Heterosis can occur due to crossbreeding, which increases the proportion of heterozygous genes (Mahmud et al. 2017). Increased carcass protein and amino acids can reduce dietary fat and increase crude protein or single amino acids.

Microbiological quality

Total Plate Count (TPC)

Microbiological analysis of IPB-D1 chicken meat determines the microbial content in the meat. The results of the average and standard deviation of the TPC test of IPB-D1 chicken meat are shown in Table 3. Analysis of microbiological quality in the form of total bacterial colonies (total plate count) of IPB-D1 chicken meat from different rearing locations and native chicken, Sentul, and broiler meat as comparisons can be seen in Table 3. Different locations of IPB-D1 chicken rearing and free-range chicken, Sentul, and broiler as a comparison significantly affected the total bacterial colonies for all of the samples compared to broiler chicken. The average yield of total bacterial colonies in all locations was 10^5 $CFUg^{-1}$ (5Log $CFUg^{-1}$). The study results showed that the TPC content in chicken meat was above the standard limit of SNI (2009), which was $1x10^6$ (6Log $CFUg^{-1}$). The same problem was also reported by Hafid et al. (2014) on broiler chickens in several traditional markets, which are about 1.7×10^7 $CFUg^{-1}$. These data indicate the possibility of contamination of chicken meat by bacteria found in the environment, contact with the equipment used, and storage temperature. Ganie et al. (2015), the level of microbial contamination in chicken meat can occur after the slaughter or when in contact with knives, meat cutting mats, or other equipment. In addition, the temperature factor and storage time are also the cause of bacterial growth. Microbial contamination of meat can occur when the animal is still alive until it is ready to be consumed. The initial contamination comes from microbes that enter the blood circulation at slaughter because the tools used are not hygienic. According to (SNI 2897:2009) the standard content of TPC in fresh, frozen (carcass and boneless), and minced chicken is not more than $1x10^6$ $CFUg^{-1}$.

Organoleptic quality

Organoleptic testing of IPB-D1 chicken meat determined the level of acceptance by consumers. The average results and standard deviation of organoleptic testing of IPB-D1 chicken meat are shown in Table 4. Some of the panelists' considerations in assessing a food ingredient include aroma, color, and taste. The aroma of meat develops during cooking and also gives the meat a distinctive taste, which is due to the fat content in the meat. Semjon et al. (2020), the factors that can affect the aroma, taste, texture, and color of poultry meat are age, sex, nation, cage environment, slaughter conditions, and water content of meat. and intramuscular fat. Analysis using Tukey's test on hedonic testing showed that there

Table 3 . Microbiological analysis of IPB-D1 meat reared at different locations and chicken, Sentul, and broiler meat for comparison

Parameter	Rearing Location						
	IPB D1 Sukabumi Chicken	IPB D1 Bekasi Chicken	D1 Sukabumi Final Stock Chicken	D1 Bekasi Final Stock Chicken	Kampong Chicken	Sentul Chicken	Broiler Chicken
Total bacterial colonies (log $CFUg^{-1}$)	6.12 ± 0.82^b	6.40 ± 0.82^b	5.41 ± 0.18^b	5.62 ± 0.39^b	5.62 ± 0.02^b	5.21 ± 0.01^b	9.00^a

Different superscripts in the same row showed significantly different effects of the 5% Tukey test ($P<0.05$)

Table 4. The organoleptic quality of IPB-D1 chicken meat reared at different locations and Kampong chicken, Sentul, and broiler meat for comparison

Test	Parameter	Rearing Location						
		IPB D1 Sukabumi Chicken	IPB D1 Bekasi Chicken	D1 Sukabumi Final Stock Chicken	D1 Bekasi Final Stock Chicken	Kampung Chicken	Sentul Chicken	Broiler Chicken
Quality Hedonic	Color	2.49±1.1^{ab}	2.29±1.2^{ab}	2.73±1.1^{a}	2.98±1.2^{a}	1.69±1.2^{a}	1.95±0.9^{b}	2.44±1.0^{ab}
	Scent	2.67±1.3^{abc}	1.95±1.0^{ab}	3.02±1.2^{bc}	2.47±1.2^{bc}	3.28±0.9^{a}	3.09±1.2^{ab}	3.20±1.2^{ab}
	Intensity Mucus	3.42±1.0	3.62±1.0	3.2±0.8	3.24±0.9	3.09±0.9	3.40±0.9	3.36±0.8
Hedonic	Color	3.20±0.8^{ab}	2.89±0.9^{ab}	3.42±0.9^{a}	3.47±0.9^{a}	3.20±0.8^{ab}	2.86±1.2^{ab}	2.66±1.2^{b}
	Scent	3.00±0.9^{ab}	2.15±0.9^{c}	3.29±0.8^{a}	2.57±1.0^{bc}	3.22±0.9^{a}	3.11±0.8^{ab}	2.84±0.8^{ab}
	Texture	3.29±0.82	3.02±0.84	3.42±1.01	3.02±0.91	3.13±0.78	3.16±1.04	3.07±1.01
	General Appearance	3.53±0.9^{ab}	3.00±0.8 b	3.58±0.8^{a}	3.47±0.9^{ab}	3.16±0.8^{ab}	3.02±1.0^{ab}	3.00±1.1^{b}

Color 1= Red; 2= Pale, unattractive; 3= White; 4= White, attractive; 5= White, very attractive. Scent 1= Very odorless meat; 2= Odorless meat; 3= Characteristic aroma of meat, there is a deviant aroma; 4= Distinctive smell of meat, no deviant aroma; 5= Distinctive meat aroma, delicious. Mucus 1= Very slimy; 2= Slimy; 3= Slightly slimy; 4= Not slimy; 5= Very not slimy. Hedonic score: 1= Very dislike; 2= Dislike; 3= Like; 4= Somewhat like; 5= Really like. Different superscripts in the same row showed significantly different effects of the 5% Tukey test (P<0.05)

was a significant difference (P<0.05) in the preference of the panelists on all attributes, namely color, aroma, and general appearance; in other words, differences in the type of chicken affected the level of preference for chicken meat, but not there was a significant difference to the texture. Observational data are in Table 4. It shows that the type of IPB-D1 chicken compared to other chickens gave a significant hedonic quality (P<0.05) on the aroma and color of the meat. However, there was no significant difference in the intensity of mucus; this is due to the different processes of maintaining and meat storing.

The sample type has a significant effect (P<0.05) on the color of chicken meat. The data showed that the panelists' preference for meat color was in the range of 2 (somewhat like) to 3 (liked), while the hedonic quality of the meat was in the range of 2 (pale, less attractive) to 4 (white, attractive). The types of chicken meat samples at different locations did not significantly affect the meat's texture. Panelists' assessment of the level of preference for meat texture ranges from 3 (like) to 4 (somewhat like). The observations showed in Table 4 that the types of chicken meat at different locations had a significant effect (P<0.05) on the aroma of the meat. Panelists' assessment of the level of preference for meat aroma was in the range of 2 (slightly like) to 3 (liked), while the hedonic quality of the meat was in the range of 2 (Odorless meat) to 4 (typical of meat, no deviant aroma). The observed data are in Table 4. It shows that the types of chicken meat samples at different locations significantly affected the intensity of the meat mucus. The hedonic quality of meat ranges from 3 (slightly slimy) to 4 (not slimy). The color of poultry meat is influenced by several factors such as age, sex, breed, cage environment, slaughter environment, conditions before slaughter, slaughter and storage conditions, intramuscular fat, the water content of meat and feed given, meat color also influenced by the water content and pH of the meat (Semjon et al. 2020). The primary texture assessment is firmness (toughness or level of tenderness), compactness, and juiciness. The cooking time and temperature can determine meat texture (Hafid 2017; Herlina et al. 2020).

CONCLUSION

The quality of IPB-D1 chicken meat and its Final Stock in terms of physical quality, such as pH and water holding capacity, had a lower cooking loss and more tenderness than Kampong, Sentul, and broiler chickens and contained high chemical quality in protein and ash. In addition, it has a high mineral content but low in cholesterol. Organoleptic testing also showed an excellent preference for panelists for IPB-D1 chicken meat. Microbiological quality analysis in the form of *total bacterial colonies* (TPC) of IPB-D1 chicken meat and its Final Stock is under the *Maximum Microbial*

Contamination Limit set by the Indonesian National Standardization Agency in 2009 (SNI 2897:2008). All these bacteria are not resistant to high temperatures, so the cooking process is still safe for consumption. Based on the results of this study, it is necessary to improve handling practices, hygienic packaging, to sanitation so that the quality of chicken meat can be improved and microbial contamination can be minimized.

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An Empirical Evaluation of Policy Options for Increasing Dairy Production in Indonesia: A System Dynamics Approach

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ABSTRAK

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Produksi susu domestik di Indonesia tumbuh lebih lambat dibandingkan konsumsi, sehingga terjadi kelebihan permintaan yang harus dipenuhi dari impor. Akselerasi peningkatan produksi susu dalam negeri tidak bisa lagi diselesaikan secara parsial, namun diperlukan pendekatan sistem secara holistik. Penelitian ini bertujuan untuk mengevaluasi secara empiris kebijakan akselerasi peningkatan produksi susu di Indonesia. Penelitian menggunakan data sekunder dari Badan Pusat Statistik, Kementerian Pertanian, Kementerian Perdagangan, Kementerian Perindustrian, Kementerian Koordinator Bidang Perekonomian, Kementerian Koperasi dan UMKM, Bank Indonesia, and FAO. Pendekatan sistem dinamik digunakan untuk membangun model dan menggambarkan dampak jangka pendek, menengah, dan panjang berdasarkan opsi skenario kebijakan. Hasil empiris menunjukkan bahwa kebijakan calf rearing pedet, peningkatan impor sapi perah betina dewasa, peningkatan conception rate, dan proteksi tarif impor berdampak positif terhadap pangsa produksi susu domestik terhadap permintaan susu. Di sisi lain, kebijakan peningkatan konsumsi susu yang jika tidak diimbangi dengan peningkatan produksi susu domestik, berdampak negatif terhadap pangsa produksi susu domestik. Semua opsi skenario kebijakan, kecuali kebijakan peningkatan laju konsumsi susu, dalam jangka panjang meningkatkan pangsa produksi susu domestik lebih tinggi dibandingkan jangka pendek dan menengah. Disimpulkan bahwa kebijakan optimis melalui calf rearing, peningkatan impor sapi betina, conception rate, dan mempertahankan tarif impor merupakan kebijakan terbaik yang berdampak pada peningkatan pangsa produksi susu domestik tertinggi dibandingkan kebijakan pesimis dan moderat.

Kata Kunci: Permintaan Susu, Produksi Susu, Analisis Kebijakan, Skenario, Sistem Dinamik

ABSTRACT

Priyono, Nurmalina R, Burhanuddin, Ilham N. 2023. An empirical evaluation of policy options for increasing dairy production in Indonesia: a system dynamics approach. JITV 28(3):208-219. DOI:http//dx.doi.org/10.14334/jitv.v28.i3.3281.

Domestic dairy production in Indonesia grows slower than consumption, resulting in an excess demand that imports must fulfill. Accelerating dairy production can no longer be solved partially; a holistic system approach is required. This study aims to empirically evaluate the policy options for accelerating dairy production in Indonesia. The data used in this study were secondary data from Badan Pusat Statistik, the Ministry of Agriculture, the Ministry of Trade, the Ministry of Industry, the Coordinating Ministry for Economic Affairs, the Ministry of Cooperatives and SMEs, Bank Indonesia, and FAO. A system dynamics approach was used to construct the model and describe the short-, medium-, and long-term impacts based on policy scenario options. The empirical results showed that the calf rearing program policy, increased female dairy cattle imports, higher conception rates, and import tariffs all positively impacted the share of domestic dairy production to dairy demand. On the other hand, a policy of increasing dairy consumption, if not accompanied by an increase in domestic dairy production, harms the domestic dairy production share. Except for the policy of increasing dairy consumption, all policy scenarios increased the long-term share of domestic dairy production more than the short- and medium-term. It is concluded that an optimistic policy through calf rearing, increased import of female cows, conception rate, and maintaining import tariffs was the best policy with the highest impact on increasing the share of domestic dairy production compared to pessimistic and moderate policies.

Key Words: Dairy Demand, Dairy Production, Policy Analysis, Scenarios, System Dynamics

INTRODUCTION

As one of the potential countries for developing the dairy industry in Southeast Asia, Indonesia is still an importer of dairy products. Currently, imports are still required to fulfill about 77.26% of dairy consumption, primarily from New Zealand, Australia, the United States, and Europe. Domestic dairy production only

supplies 997.35 thousand tons of the 4,385.73 thousand tons of dairy demand in 2020 (Badan Pusat Statistik 2021). Moreover, the growth rate of domestic dairy production in Indonesia is lower than that of dairy consumption (Pusdatin 2020). Therefore, Indonesia's dairy cow population still needs to be developed because there is still a gap between dairy production and demand.

Indonesia faces challenges and opportunities in accelerating dairy supply from upstream to downstream to reach the target domestic dairy production share to dairy demand. The rise of dairy production depends on dairy cow productivity and population growth. During 1980-2019, the population of dairy cows in Indonesia grew by 3.01%/year, while productivity grew by 1.71%/year, and milk production grew by 4.34%/year (Priyono et al. 2022). On the other hand, the average dairy consumption in Indonesia is predicted to increase along with the increase in income and public awareness of consuming dairy products. Therefore, the gap between domestic dairy production and demand will continue unless domestic dairy production accelerates.

The government has issued several policies to develop the dairy industry in Indonesia. The 2013–2025 Indonesian Dairy Blueprint was launched by the government through the Coordinating Ministry for Economic Affairs in 2014 and then reviewed in 2016. The target share of domestic dairy production reached 60% of dairy demand. Furthermore, the Ministry of Agriculture issued Regulation No. 26/2017 concerning dairy supply and distribution after revoking Presidential Instruction No. 2/1985. Hereafter, the Minister of Agriculture regulation was revised to become No. 33/2018 to respond to World Trade Organization (WTO) policy regulations. The government previously developed a dairy industry roadmap for 2010–2025 through the Ministry of Industry, Republic of Indonesia. The long-term target output in the roadmap includes increasing domestic dairy production to 50–60% through increasing dairy productivity and dairy cow population. Therefore, improving and developing the agribusiness system from upstream to downstream is important in achieving the target share of domestic dairy production, about 60% of dairy demand, while supporting Indonesia's vision as the world's food barn in 2045.

The acceleration of domestic dairy production faces several problems, especially in the upstream and on-farm subsystems. According to Susanty et al. (2019), the productivity of dairy cows in Indonesia has stagnated at 8 to 12 liters/head/day, and cow ownership is 2 to 3 heads per household. Furthermore, the deficit of land-carrying capacity in the dairy cow population centers also needs to be improved to fulfill the required feed (Parmawati et al. 2018). Regarding reproductive performance, a survey on livestock business costs showed that the conception rate of dairy cows in Indonesia is 40.57% (Badan Pusat Statistik 2017). Moreover, the conception rate and the success of the rearing calf program will significantly affect the increase in the dairy cow population and domestic dairy production. According to Ferguson and Skidmore (2013) and Siddiqui et al. (2013), reproductive management and rearing programs aim to increase fertility and the lactating dairy cows' population. Therefore, based on the existing conditions, conception rates, and rearing programs can be implemented to encourage increased dairy production in Indonesia.

Based on data from the Ministry of Agriculture and Badan Pusat Statistik, the level of dairy consumption in Indonesia in 2000, 2005, 2010, 2015, and 2020 were 6.4, 9.3, 13.2, 15.0, and 16.27 liters/capita/year, respectively. Furthermore, if a rise in domestic dairy production does not accompany an increase in dairy consumption, it will decrease the share of domestic dairy production to dairy demand. Moreover, the government implements an import tariff policy as a trade barrier that can be used as an import protection policy for dairy producers. In addition, these intervention policies aim to protect dairy farmers (Saptati and Priyono 2021) and encourage an increase in domestic production (Salvatore 2013).

Based on the existing conditions, to achieve 60% of the target domestic dairy production share to dairy demand, it is necessary to accelerate the increase in milk production at the farmer level. Most Indonesian dairy producers are smallholder farms, owning less than 4 cows per household (Susanty et al. 2019; Saptati and Priyono 2021). The target of accelerating domestic dairy production can be realized if the development of dairy cows in the upstream and on-farm subsystems can be strengthened. Problems with increasing dairy production in Indonesia could no longer be solved partially; instead, a holistic system approach was required. The system dynamics approach can be used to build an integrated interactive model to increase the availability of dairy supply in Indonesia. Therefore, the empirical evaluation of policy options for accelerating dairy production in Indonesia using a system dynamics approach must be conducted holistically. This study aims to empirically evaluate the policy options proposed for accelerating the increase in the share of domestic dairy production to dairy demand in Indonesia.

MATERIALS AND METHODS

Data collection

This study employed official quantitative data sources from Badan Pusat Statistik, the Ministry of Agriculture, the Ministry of Trade, the Ministry of Industry, the Coordinating Ministry for Economic Affairs, and Ministry of Cooperatives and SMEs Republic of Indonesia, Bank Indonesia, and the Food and Agriculture Organization of the United Nations (FAO).

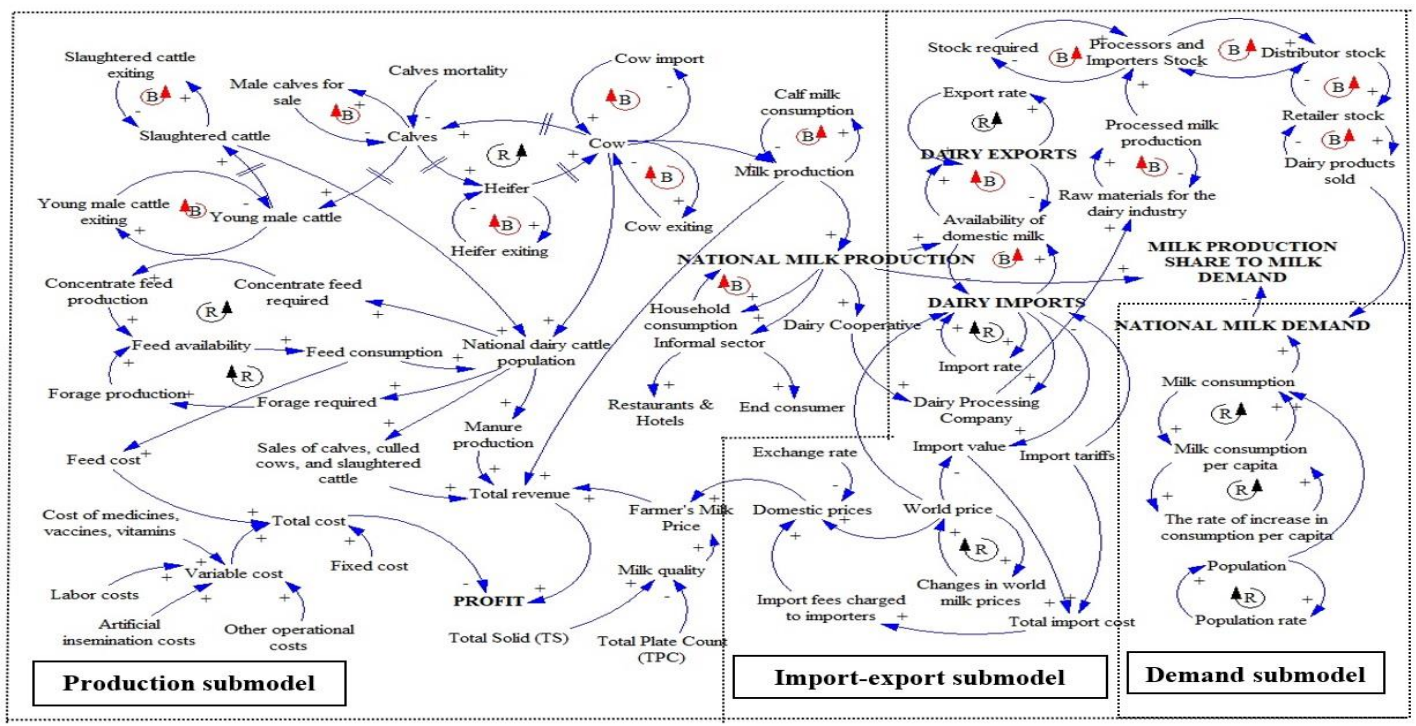

Figure 1. Causal loop diagram of dairy supply model in Indonesia

Table 1. Policy scenario options for accelerating dairy production in Indonesia for the period 2020-2045

Scenario*	Pessimistic Policy	Moderate Policy	Optimistic Policy
Scenario 1: (*Calf rearing and increasing cow imports*)	2.66%/year	20%/year	35%/year
Scenario 2: (*Increasing the conception rate*)	5%	15%	25%
Scenario 3: (*The increasing dairy consumption rate*)	4.5%/year	5.5%/year	6.5%/year
Scenario 4: (*Import Tariff*)	5%	10%	10%
Scenario 5: (*Combination of scenarios 1 and 2*)	2.66%/year & 5%	20%/year & 15%	35%/year & 25%
Scenario 6: (*Combination of scenarios 1, 2, and 3*)	2.66%/year; 5%; & 4.5%/year	20%/year; 15%; & 5.5%/year	35%/year; 25%; & 6.5%/year
Scenario 7: (*Combination of scenarios 1, 2, 3, and 4*)	2.66%/year; 5%; 4.5%/year; & 5%	20%/year; 15%; 5.5%/year; & 10%	35%/year; 25%; 6.5%/year; & 10%

*Policy scenario options will be implemented starting in 2025–2045; Short-term= 2025-2030; Medium-term= 2025-2035; Long-term= 2025-2045

Secondary data were collected at the national level, including the population of dairy cows, dairy production, import and export of dairy products, dairy consumption, domestic dairy prices, imported dairy prices, exchange rates, and related supporting variables. The other quantitative data used in the model development include data from farmers, dairy cooperatives, GKSI, and samples of dairy processing companies obtained from research and development activities at the Indonesian Center for Animal Research and Development, IAARD, Ministry of Agriculture. Primary data were used to confirm the national-level data, which included four dairy cooperatives in West Java, five dairy cooperatives and one milk processing plant in Central Java, and five dairy cooperatives in East Java. The collected quantitative data were used to fill in all the variables in the model, which was developed through a stock and flow diagram. The validation of the model structure involved lecturers, researchers, and practitioners using a purposive sampling technique with the criteria of being experts in developing the dairy industry in Indonesia.

Data analysis method

This paper employed a system dynamics approach to building a dairy supply model. The model was created in three sub-models: production, import-export, and consumption. The stages of system dynamics method analysis consist of problem formulation, system identification, model formulation, model validation, and policy simulation (Forrester 1994; Lie et al. 2018; Susanty et al. 2019; Simões et al. 2020; Azizsafaei et al. 2022). The dairy supply model in this study is described in more detail in a causal loop diagram. According to Sterman (2000), causal loop diagrams provide graphical information about the interrelated relationships between elements in the model. The upstream arrow indicates the cause, and the end of the arrow shows the effect with a positive or negative sign. A causal loop diagram of the dairy supply model in this study can be seen in Figure 1.

Furthermore, causal loop diagrams were developed in stock and flow diagrams. Quantitative data was inputted into stock and flow diagrams on models that passed structural validity testing stages. The validity test of the structure model was conducted to test the model's beliefs and the interaction of causal variables to approach the structure of real-life phenomena. Furthermore, a mean absolute percentage error (MAPE) statistical test was used for output validation. MAPE is a statistical test measuring the simulation output's accuracy. According to Sterman (2000), the mean absolute error (MAE) value between the actual data value and the simulation can be used as an error test tool for simulation results with actual conditions. The formula to calculate MAPE can be seen below:

$$MAPE = \frac{1}{n}\sum_{t=1}^{n}\left(\frac{|Y_t - \overline{Y}_t|}{Y_t}\right) x\ 100$$

Where Y_t is actual data values, $\overline{Y}_t$ is model simulation values, and n is year/time interval.

The model is valid for simulation if the MAPE is small and the deviation between the simulation output and actual data is also small. Furthermore, the model is simulated in compliance with the simulation objectives after being tested for validity (Forrester 1994; Sterman 2000). The causal loop diagram in this study was created using Vensim PLE. Furthermore, stock and flow diagram development, validation testing, and model simulation were performed using Powersim Studio 10. The simulation analysis performed in the 2020–2045 period and the detailed policy scenario options in this model can be seen in Table 1.

RESULTS AND DISCUSSION

Model validation

Model validation was an essential step in system dynamics methodology involving quantitative and qualitative tools. The model validation in this study used dairy cow population, dairy production, and dairy products import variables for the 2015–2019 periods. According to Sterman (2000), mean absolute error (MAE) is used to evaluate models and measure forecast accuracy. The result showed that the mean absolute percentage error of the dairy cow population, dairy production, and dairy product import variables were 3.85%, 3.67%, and 7.16%, respectively. Values of MAPE below 10% designate high accuracy in prediction. As a result, the model in this study met the goodness of fit requirement for policy simulation.

Calf rearing program and higher conception rate policy

Good dairy farming practices, professional farm management, and superior nutrition are required for profitable dairy farming. Dairy farmers frequently suffer profit losses due to delayed sexual maturation and higher age at first calving (Do et al. 2013; Wathes et al. 2014). Therefore, it is critical to manage calves at all stages of growth so that calves grow at the desired rate. Imported superior cows with superior genetics and high productivity should have been included in the program to improve calf-rearing outcomes. Figure 2 shows the impact of the calf rearing program and higher conception rate policy on the dairy cow population. The study found that calf-rearing programs and increased dairy cow imports (scenario 1) positively impacted dairy cow

population growth. Scenario 1 led to a higher dairy cow population than the baseline under pessimistic, moderate, and optimistic policies.

A good fertility management plan is required to ensure that all actions are directed toward improving reproductive performance. According to Boulton et al. (2017) and Kim and Jeong (2019), the rate of conception and calving will be determined by successful reproductive management, which includes calf and heifer rearing, first mating, pregnancy, and calving. The study also discovered that the conception rate positively impacted the dairy cow population. Higher conception rates (scenario 2) also led to a higher dairy cow population than the baseline, both pessimistic, moderate, and optimistic policies. The conception rate is the percentage of successful inseminations that result in pregnancy. To determine the percentage of conception rate, the insemination date or natural mating date must be collected. The conception rate is affected by the length of the waiting period, heat detection, mating method, body condition score, and feed intake (Siddiqui et al., 2013; Dash et al., 2016; Kim and Jeong, 2019). It is concluded that the policy of the calf rearing program, improved genetics and productivity, and a higher conception rate will lead to a higher dairy cow population.

Dairy import protection policy

The government enacts restrictive trade policies to protect domestic industries. According to Minister of Finance Regulation No. 26 of 2022, the dairy import tariff in Indonesia is fixed at 5%. The study showed that increasing import tariffs from 5% to 10% would reduce the volume of dairy imports (Figure 3). On the other hand, the reduction in import tariffs from 5% to 0% will result in increased import volumes. Based on the simulation results for 2020–2045, the trend for the baseline import rate was 8.26% per year. The 10% import tariff scenario decreased the import volume rate to 5.74% per year. Conversely, a 0% import tariff increased the rate of import volume to 10.42% per year.

The results showed that an increased import tariff of 5% impacted a 6.05% share of domestic dairy production, higher than the baseline. Based on the results, it was indicated that there is a correlation between import tariff, import volume, and dairy production. According to Salvatore (2013); Shagdar and Nyamdaa (2017), import tariffs are restriction policies that protect farmers by creating competitive and profitable domestic agricultural commodity prices. Import tariffs on dairy products have become necessary since the non-tariff policy was abolished in Indonesia. The government implements import tariff protection in the dairy industry by imposing import fees or taxes on dairy products imported from other countries. As a result of the increase in import tariffs, the total dairy import volume will decrease. Therefore, an import tariff will protect dairy farmers and decrease dairy import volume.

The increase in dairy consumption policy

Increasing dairy consumption is an action plan in the Indonesian dairy blueprint stipulated by the Coordinating Ministry for Economic Affairs. The results showed that household dairy consumption in Indonesia increased positively, with a growth rate of 3.63% per year during 2002–2019. Furthermore, the population growth rate in Indonesia was 1.43% per year (2000–2019), and the gross domestic product increased by 5.07% per year (2010–2019). Dairy consumption has positively increased along with population growth, increased income, and improved socio-economic conditions in consumer households (Akaichi and Revoredo-Giha 2012; Lagrange et al. 2015; Cheng et al. 2015).

Figure 4 shows the impact of policy options in different scenarios on the share of domestic dairy production. The results showed that an increase in higher dairy consumption (optimistic policy) impacted decreasing the higher share of domestic dairy production compared to a pessimistic and moderate policy (scenario 3 in Figure 4). A comparison of the impact of Scenario 5 (without increasing dairy consumption policy) with Scenario 6 (increasing dairy consumption policy) showed that the share of domestic dairy production in Scenario 6 was lower than in Scenario 5. It is clear that the policy of dairy consumption, if not accompanied by an increase in domestic dairy production, harms domestic dairy production.

Impact of pessimistic policy scenario on domestic dairy production

Table 2 shows the changes in domestic dairy production share to dairy demand for pessimistic policy over the short-, medium-, and long-term compared to the baseline. The calf rearing program and increased cow import by 2.66 %/year (Scenario 1) increased the share of domestic dairy production to dairy demand by 2.07% to 5.82% higher than baseline. An increase in the conception rate by 5% (Scenario 2) increased the share of domestic dairy production by 0.11% to 4.87% compared to the baseline. Scenarios 1, 2, 3, and 4 showed that Scenario 1 was the best Scenario, which increased the highest share of domestic dairy production in the long term (Table 2). However, the results of all policy scenario options showed that Scenario 5 led to the highest increase in the share of domestic dairy production by 2.18% to 10.98% in the long term.

In contrast, a program to boost dairy consumption (Scenario 3) has an impact of decreasing the domestic

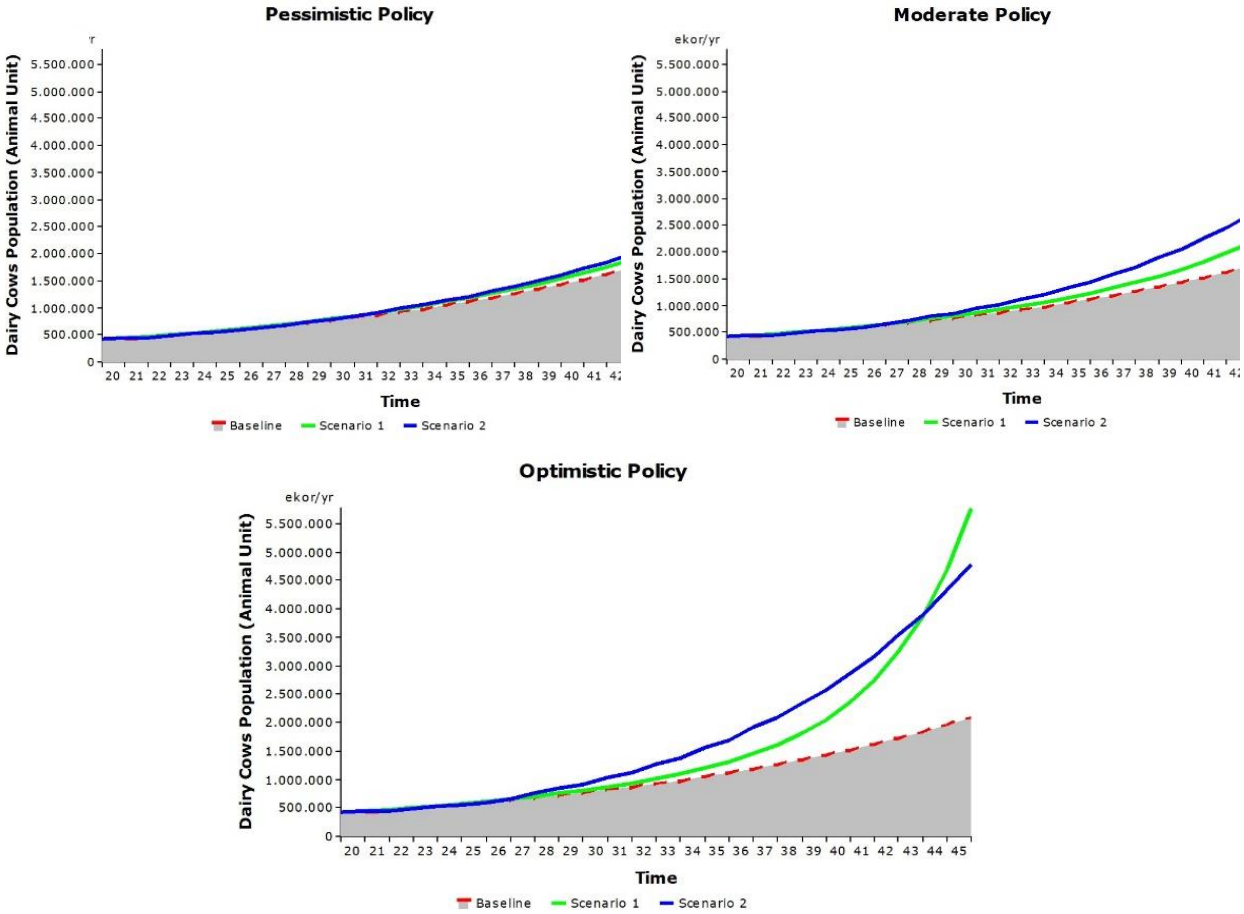

Figure 2. The impact of calf rearing program and higher conception rate policy on dairy cow population in Indonesia for the period 2020–2045

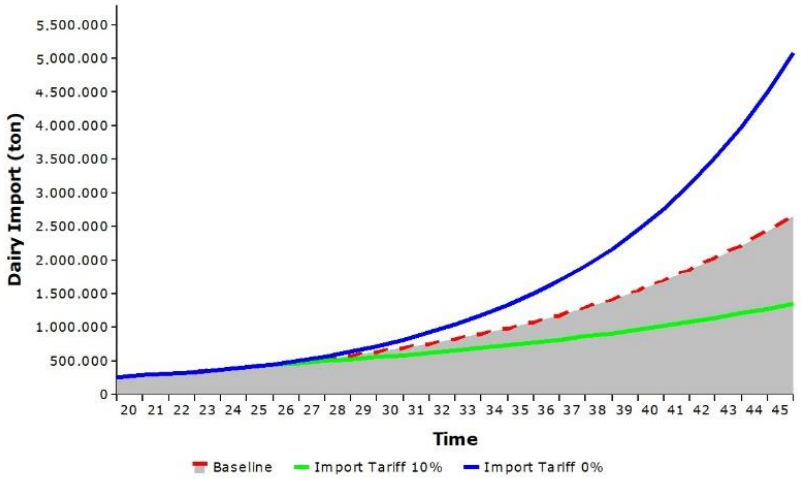

Figure 3. The impact of import tariff on dairy import volume in Indonesia for the period 2020–2045

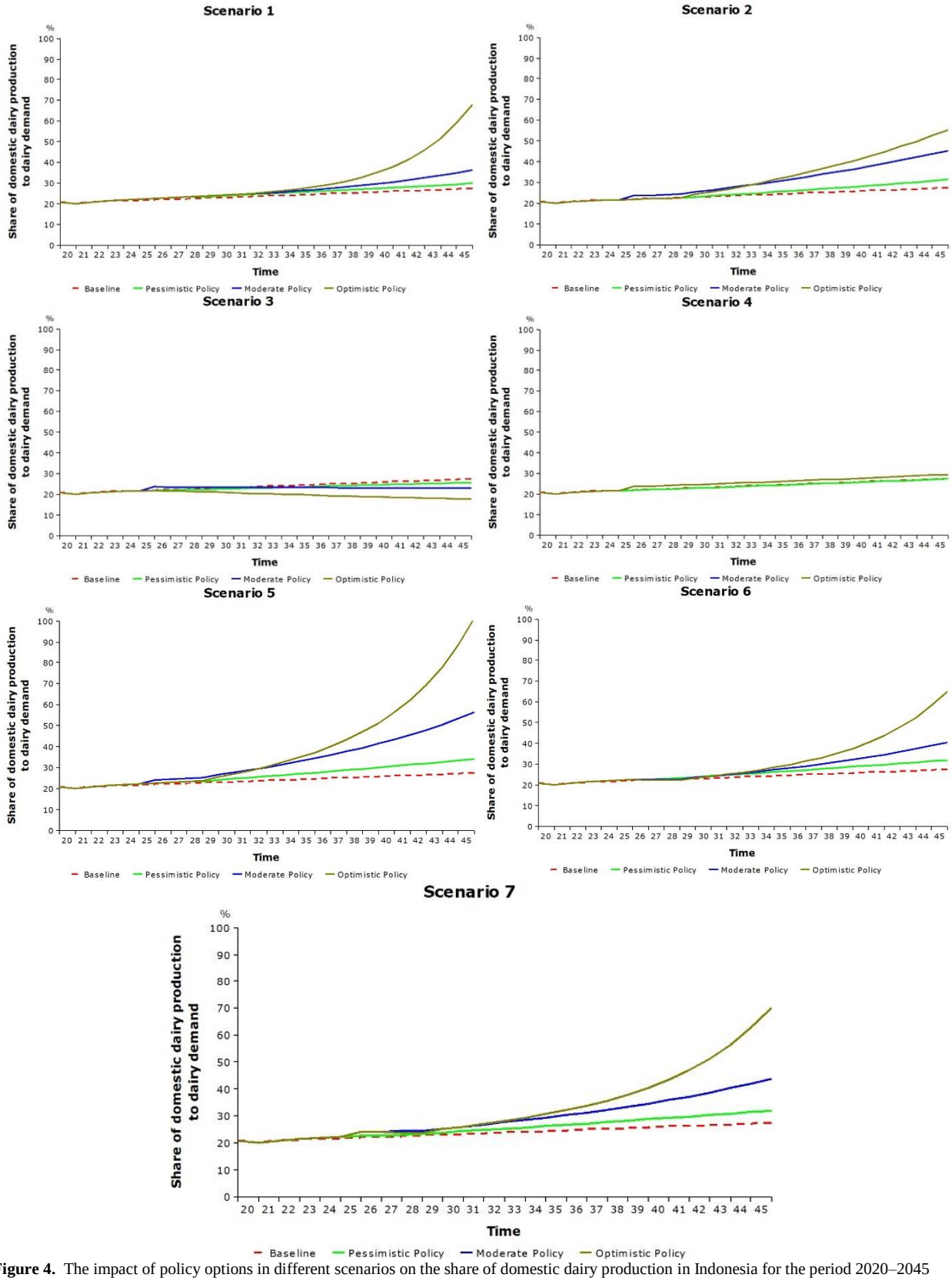

Figure 4. The impact of policy options in different scenarios on the share of domestic dairy production in Indonesia for the period 2020–2045

Table 2. The impact of pessimistic policy options scenario on the share of domestic dairy production in Indonesia for the short, medium, and long-term

	Short-term	Medium-term	Long-term
	Change (%)*	Change (%)*	Change (%)*
Scenario 1: *(Calf rearing + increasing cows import by 2.66%/year)*	2.07	3.53	5.82
Scenario 2: *(Increasing the conception rate by 5%)*	0.11	1.23	4.87
Scenario 3: *(The increasing of dairy consumption rate by 4.5%/year)*	-0.30	-0.93	-2.43
Scenario 4: *(Import Tariff by 5%)*	0.00	0.00	0.00
Scenario 5: *(Combination of scenarios 1 and 2)*	2.18	4.83	10.98
Scenario 6: *(Combination of scenarios 1, 2, and 3)*	1.87	3.82	8.11
Scenario 7: *(Combination of scenarios 1, 2, 3, and 4)*	1.87	3.82	8.11

*Percentage change relative to baseline. Short-term = 2025-2030; Medium-term = 2025-2035; Long-term = 2025-2045

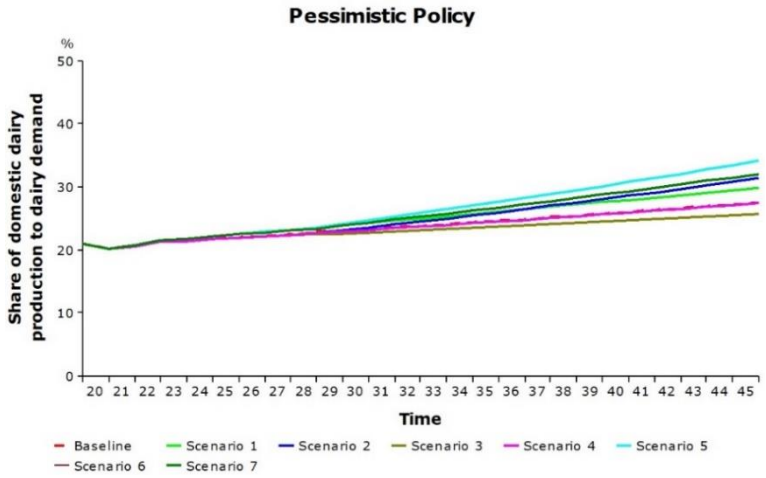

Figure 5. Dynamic simulation result of pessimistic policy scenario in Indonesia for the period 2020–2045

dairy production share by 0.30 to 2.43% in the long term. Under current conditions, the government of Indonesia regulates dairy drinking programs and school milk programs to promote an increase in dairy consumption. According to Rabiei et al. (2021), dairy consumption can be increased by introducing training programs for children, adolescents, and adults, providing more healthy and attractive dairy products, and lowering prices. Modern food marketing methods also effectively increase dairy consumption and build customer trust in milk quality (Cheng et al. 2015).

In the baseline for pessimistic policy, the share of domestic dairy production to dairy demand increased from 20.24% in 2020 to 27.64% in 2045 (Figure 5). Except for scenario 3, the share of domestic dairy production to dairy demand in scenarios 1-7 was higher than the baseline. Based on the simulation result, increasing the conception rate by 5% (Scenario 2) and increasing cow imports by 2.66 %/year (Scenario 1) revealed similar shares of domestic dairy production from 2025–2037. However, beginning in 2038, the share of domestic dairy production for Scenario 2 was higher than for Scenario 1. However, the combination of Scenarios 1 and 2 (Scenario 5) revealed a higher share of domestic dairy production to dairy demand than the baseline and all policy scenario options. As a result, in the pessimistic policy, the calf rearing program, higher cow imports, and higher conception rates simultaneously positively impact the share of domestic dairy production to dairy demand. According to Russell et al. (2022), implementing calf rearing needs to consider several factors, including calf growth, health, behavior, management practices, the rearing environment, equipment, and external advice. To increase the conception rate, dairy farmers need extensions and training in feed nutrition and reproductive management (Siddiqui et al., 2013).

Impact of moderate policy scenario on domestic dairy production

There is a distinction between pessimistic and moderate policies. In the moderate policy (Table 3), an increase in the conception rate by 15% (Scenario 2)

showed a higher share of domestic dairy production than increasing cow imports by 20% per year (Scenario 1). Scenario 2 showed a lower increase in the short term than scenario 1. However, in the long term, Scenario 2 increased the share of domestic dairy production above Scenario 1. Furthermore, scenario 5 raised the share of domestic dairy production to dairy demand by 2.47% to 29.27%. Therefore, Scenario 5 delivered the best short-, medium-, and long-term performance compared to the baseline and other policy scenario choices. The calf rearing program, followed by an increase in the conception rate and superior cow imports (Scenario 5), aims to increase dairy productivity and domestic dairy production. Genetics and breeding are determinants of milk yield per lactating cow (Lima et al., 2022), and proper nutrition in feeding lactating cows affects milk production significantly (Tramontini et al., 2021). Therefore, the program for calf rearing must be supported by enhancing sound reproduction and rearing management. Costa et al. (2022) state that effective management, milking hygiene practices, and milk storage management determine higher dairy volume.

In the moderate policy, the increase in dairy consumption (Scenario 3) lowered the share of domestic dairy production by a higher amount (from -1.19% to -9.18%) than in the pessimistic policy (from -0.30% to -2.43%). On the other hand, an increase in import tariffs (scenario 4) showed an increase in the domestic dairy production share from 3.59% to 6.05%. According to Salvatore (2013), import tariffs on small countries (partial equilibrium) increase domestic production. Forty countries of the World Trade Organization (WTO) have also implemented agricultural tariff-rate quotas. For instance, Mongolia imposes import tariffs on its WTO-bound rates to encourage domestic industries (Shagdar and Nyamdaa 2017). Based on all policy scenarios options in this study, scenario 7 is the best Scenario with the highest impact on increasing domestic dairy production share to dairy demand in the short- and medium-term. However, in the long term, scenario 5 has the highest increase in the share of domestic dairy production.

Even though scenario 5 was the best Scenario for raising domestic dairy production's share of dairy demand under the moderate policy, the government's commitment to boosting dairy consumption (scenario 6) revealed that the share of domestic dairy production was lower than scenario 5 (Figure 3). Figure 3 further proved that increasing import tariffs (scenario 4) increased the share of domestic dairy production, as indicated by the line in scenario 4 being higher than the baseline line. The result showed that scenario 7 increased the share of domestic dairy production to 43.89% in 2045. The comparison of Figures 5 and 6 also showed that increased dairy consumption impacted the gap line between scenario 3 and the baseline, which was more significant than the gap line for the pessimistic policy.

Impact of optimistic policy scenario on domestic dairy production

In the short term, the optimistic policy resulted in a tremendous increase in domestic dairy production share under Scenario 1 than in Scenario 2. In the medium and long term, however, an increase in the conception rate (scenario 2) impacts dairy production more than an increase in cow imports (scenario 1). Consequently, it is essential to enhance reproductive efficiency. Shalloo et al. (2014) state that inadequate reproductive management contributes to rising dairy farming expenses. In the long term, the combination of scenarios 1 and 2 (scenario 5) increased domestic dairy production by 61.97 %, which was much higher than scenario 1 (30.74%) and scenario 2 (27.73%) (Table 4). Scenario 7 showed an increase in the share of domestic dairy production by 38.03% in the long run.

Table 3. Impact of moderate policy options scenario on the share of domestic dairy production in Indonesia for the short, medium, and long-term

	Short-term	Medium-term	Long-term
	Change (%)*	Change (%)*	Change (%)*
Scenario 1: *(Calf rearing + increasing cows import by 20%/year)*	2.10	4.07	10.96
Scenario 2: *(Increasing the conception rate by 15%)*	0.35	4.08	16.96
Scenario 3: *(The increasing of dairy consumption rate by 5.5%/year)*	-1.19	-3.63	-9.18
Scenario 4: *(Import Tariff by 10%)*	3.59	4.92	6.05
Scenario 5: *(Combination of scenarios 1 and 2)*	2.47	8.36	29.27
Scenario 6: *(Combination of scenarios 1, 2, and 3)*	1.21	4.08	15.37
Scenario 7:*(Combination of scenarios 1, 2, 3, and 4)*	4.89	9.32	22.60

*Percentage change relative to baseline. Short-term = 2025-2030; Medium-term = 2025-2035; and Long-term = 2025-2045

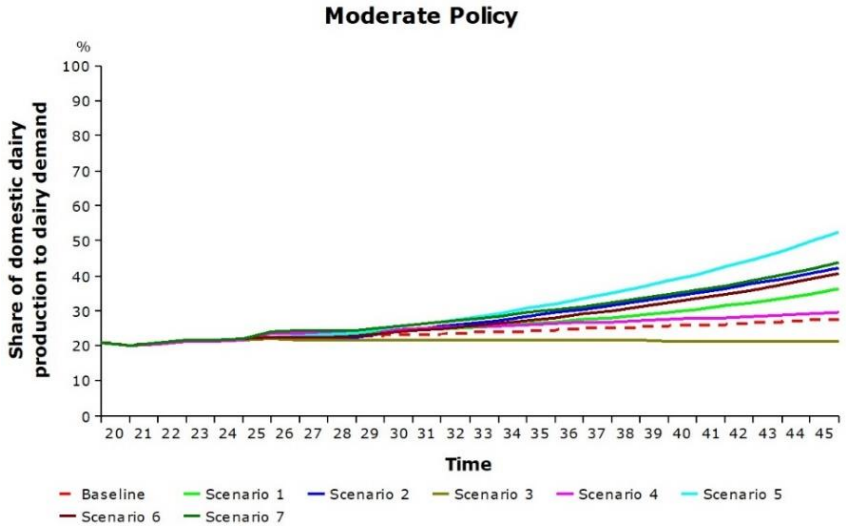

Figure 6. Dynamic simulation result of moderate policy scenario in Indonesia for the period 2020–2045

Table 4. Impact of optimistic policy options scenario on the share of domestic dairy production in Indonesia for the short, medium, and long-term

	Short-term	Medium-term	Long-term
	Change (%)*	Change (%)*	Change (%)*
Scenario 1: *(Calf rearing + increasing cows import by 35%/year)*	2.14	4.80	27.73
Scenario 2: *(Increasing the conception rate by 25%)*	0.60	7.00	30.74
Scenario 3: *(The increasing of dairy consumption rate by 6.5%/year)*	-2.04	-6.14	-15.09
Scenario 4: *(Import Tariff by 10%)*	3.59	4.92	6.05
Scenario 5: *(Combination of scenarios 1 and 2)*	2.76	12.15	61.97
Scenario 6: *(Combination of scenarios 1, 2, and 3)*	0.56	4.34	29.71
Scenario 7:*(Combination of scenarios 1, 2, 3, and 4)*	4.20	9.60	38.03

*Percentage change relative to baseline. Short-term = 2025-2030; Medium-term = 2025-2035; and Long-term = 2025-2045

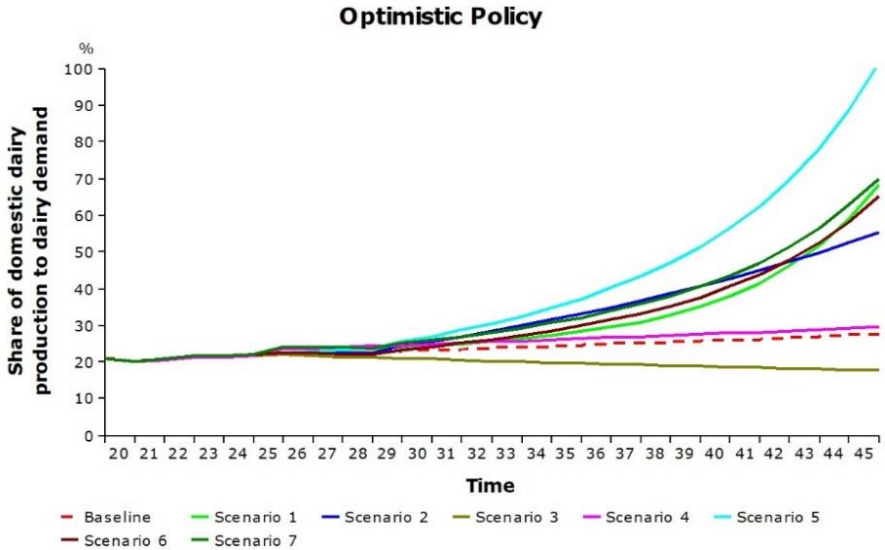

Figure 7. Dynamic simulation result of optimistic policy scenario in Indonesia for the period 2020–2045

The research findings indicated that the increase in dairy consumption combined with other scenarios in Scenario 7 has led to a decrease in the share of domestic dairy production, which was previously at 61.97% in Scenario 5 and has now decreased to 38.03% in Scenario 7. The increase in dairy consumption, assuming a lower growth rate of domestic dairy production than dairy consumption, has reduced the share of domestic dairy production; this indicates that the higher increase in dairy consumption has caused a decline in the share of domestic dairy production in response to dairy demand. As a result of excess demand, higher imports of dairy products are required to meet dairy demand (Salvatore 2013).

Furthermore, the results indicated that scenario 5 raised domestic dairy production in the long term by 17.35 times compared to the short term and in the medium term by 4.9 times compared to the short term. In comparison, scenario 7 showed an increase in domestic dairy production share in the long-term, which was 7.69 times higher than the short-term, and in the medium-term, which was 2.02 times higher than the short-term. It was determined that scenario 5 was more effective in accelerating the share of domestic dairy production to meet dairy demand. However, in the optimistic policy, scenario 7 was the best short-term Scenario, while Scenario 5 was the best medium- and long-term Scenario.

In contrast to the pessimistic and moderate policies, the optimistic policy showed a high share of domestic dairy production to dairy demand. In the optimistic policy, a rise in the conception rate (scenario 2) increased the domestic dairy production share to 53.64% in 2045, while a calf-rearing program accompanied by an increase in cow imports (Scenario 1) increased the domestic dairy production share to 64.39% in 2045. According to Bilkis et al. (2016), the conception rate is determined by the season, breed of cattle, time of insemination, and quality of the semen. In addition, the genetic improvement of dairy cows will determine dairy production and productivity (de Vries and Marcondes, 2020).

The analysis indicated that an increase in higher dairy consumption (scenario 3) resulted in a decrease in the share of domestic dairy production, which was more significant in the optimistic policy than in the pessimistic and moderate policies. Based on simulation results, scenario 5 was the best policy option among all scenarios, as shown by the sharp increase in the scenario 5 line compared to other scenarios (Figure 4). However, implementing a higher consumption policy in the optimistic policy (Scenario 7) decreased the share of domestic dairy production compared to Scenario 5.

It is concluded that the calf rearing program, enhanced female dairy cattle import policy, and higher conception rates positively impacted the share of domestic dairy production in the pessimistic, moderate, and optimistic policies. Maintaining import tariffs as a restrictive policy to protect the sustainability of domestic dairy production could also be an option to increase the share of domestic dairy production. On the other hand, policies aimed at increasing dairy consumption had the opposite effect on the share of domestic dairy production. The existing condition in Indonesia, in which the growth rate of dairy consumption is higher than that of domestic dairy production, means that implementing Scenario 3 (higher dairy consumption) resulted in a decrease in the share of domestic dairy production. Therefore, the policy of increasing dairy consumption must be balanced with the policy of increasing domestic dairy production.

CONCLUSION

It was found that the calf rearing program, enhanced female dairy cattle import policy, higher conception rates, and import tariff policy have all led to a significant increase in the share of domestic dairy production to dairy demand. The increase in dairy consumption had the opposite effect on the domestic dairy production share. In the long run, an optimistic policy was the best because it increased the highest population of dairy cows and domestic dairy production compared to pessimistic and moderate policies. The findings suggest and recommend that the government and stakeholders can implement calf rearing, enhance reproductive management, maintain import restriction policies, and carefully consider the costs and benefits of different policy options and scenarios to determine policy priorities.

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