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PREFACE

In this edition, Volume 28 No 2, 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:
"The 84-bp Indel Polymorphism of The Sterol Regulatory Element-Binding Protein 1 (SREBP1) Gene in Several Cattle Breeds in Indonesia"; "Changes in the qualitative composition of the milk of Holstein cows during summer chronic heat stress"; "Association of DGAT1 Gene Related to Flavor, Odor, Cholesterol, and Mineral in Indonesian Sheep"; "Production Performance and Sperm Characteristics of Quail (*Coturnix-coturnix japonica*) with Different Concentrations of Yolk Immunoglobulin"; "Effect of Zinc Supplementation in The Diet on Sikumbang Janti Female Duck Performance, Carcass, Digestive Organs, and Intestinal Morphology"; and "Morphological Characterization of Doe Kacang Goat in the Dry Land Area".

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, June 2023

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LIST OF CONTENT

The 84-bp Indel Polymorphism of The Sterol Regulatory Element-Binding Protein 1 (SREBP1) Gene in Several Cattle Breeds in Indonesia

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ABSTRAK

Anwar S, Putra WPB, Khaerunnisa I, Wulandari AS, Prihatin KW, Sutikno. 2023. Polimorfisme indel 84-bp gen *Sterol Regulatory Element-Binding Protein 1* (*SREBP1*) pada beberapa bangsa sapi di Indonesia. JITV 28(2):102-111. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3-3046.

Gen *Sterol regulatory element-binding protein 1* (*SREBP1*) merupakan gen pengkode SREBP1, salah satu famili faktor transkripsi yang memiliki peran kunci dalam homeostasis lipid serta metabolisme asam lemak. Penelitian ini bertujuan untuk mendeteksi polimorfisme indel 84-bp di daerah intron 5 gen SREBP1 (84-bp indel) pada beberapa bangsa sapi di Indonesia. Hasil penelitian ini berguna dalam seleksi genetik berbasis molekuler untuk produksi daging sehat pada sapi. Penelitian ini menggunakan total 740 ekor dari enam bangsa sapi (Limousin, Simmental, Friesian Holstein, Bali, Sumbawa dan Pasundan) yang berasal dari dua Balai Inseminasi Buatan Nasional dan peternak rakyat. Polimorfisme 84-bp indel dideteksi menggunakan metode polymerase chain reaction (PCR) dan divisualisasikan menggunakan sistem elektroforesis gel agarosa. Hasil penelitian menunjukkan alel L (tipe insersi) merupakan alel yang umum dan ditemukan terfiksasi pada sapi Holstein-Friesian, Bali dan Sumbawa (1,00). Sedangkan alel S (tipe delesi) ditemukan pada sapi Limousin, Simmental dan Pasundan dengan frekuensi berturut-turut 0,24; 0,16 dan 0,01. Keberadaan alel S pada sapi Pasundan hanya ditemukan pada genotipe heterozigot LS (0,03). Kesimpulannya, gen *SREBP1* (84-bp indel) ditemukan dalam kondisi polimorfik pada sapi Limousin, Simmental dan Pasundan, tetapi monomorfik pada sapi Friesian-Holstein, Bali dan Sumbawa.

Kata Kunci: Asam Lemak, Indel, Sapi Lokal Indonesia, Gen *SREBP1*

ABSTRACT

Anwar S, Putra WPB, Khaerunnisa I, Wulandari AS, Prihatin KW, Sutikno. 2023. The 84-bp Indel Polymorphism of *Sterol Regulatory Element-Binding Protein 1* (*SREBP1*) Gene in Several Cattle Breeds in Indonesia. JITV 28(2):102-111. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3-46.

Sterol regulatory element-binding protein 1 (*SREBP1*) gene is a gene that encodes SREBP1, a family of transcription factors that have a key role in lipid homeostasis as well as fatty acid metabolisms. The present study aimed to detect the 84-bp indel polymorphism in the intron 5 of the SREBP1 gene (84-bp indel) in several cattle breeds in Indonesia. A total of 740 cattle of six breeds (Limousin, Simmental, Holstein-Friesian, Bali, Sumbawa, and Pasundan) from two National Artificial Insemination Centers (NAICs) and smallholder farmers were used in this study. The detection of 84-bp indel polymorphism was performed using the polymerase chain reaction (PCR) method and visualized through a gel agarose electrophoresis system. The study showed that the L allele (insertion-type) was common and is fixed in Holstein-Friesian, Bali, and Sumbawa cattle (1.00). Meanwhile, the S allele (deletion-type) was found in Limousin, Simmental, and Pasundan cattle with a frequency of 0.24, 0.16, and 0.01, respectively. The presence of the S allele in Pasundan cattle was only found in the form of the heterozygous LS genotype (0.03). In conclusion, the 84-bp indel of the SREBP1 gene was found to be polymorphic in Limousin, Simmental, and Pasundan cattle, but monomorphic in Holstein-Friesian, Bali, and Sumbawa cattle.

Key Words: Fatty Acid, Indel, Indonesian Local Cattle, *SREBP1* Gene

INTRODUCTION

The *sterol regulatory element-binding proteins* (SREBPs) are transcription factors that play a key role in lipid homeostasis (Eberlé et al. 2004). These transcription factors are recognized as master regulators of cholesterogenesis and lipogenesis in mammals since their expression was found to influence transcriptional activation in more than 30 genes encoding enzymes for cholesterol, fatty acid (FA), triglyceride (TG), and phospholipid synthesis (Eberlé et al. 2004). The SREBPs bind to sterol regulatory elements (SREs) in the promoter regions of target genes to stimulate transcription (Shimano & Sato 2017).

There are three SREBP isoforms, including SREBP1a, SREBP1c, and SREBP2 (Bionaz et al. 2020). SREBP1a and 1c regulate genes involved in fatty acid and TG synthesis, while SREBP2 primarily activates genes involved in cholesterol biosynthesis (Deng et al. 2014). Furthermore, both SREBP1a and 1c are encoded by the same gene (*SREBP1*) but their transcripts are produced from alternative splicing that differs in the first exon (exon 1a and exon 1c) (Eberlé et al. 2004). However, SREBP1c is the predominant SREBP subtype expressed in most animal tissues (Deng et al. 2014).

The *sterol regulatory element-binding transcription protein 1* (*SREBP1*) gene has several synonyms: *ADD1*, *SREBF1*, and *SREBP-1* gene (NCBI, 2021). The polymorphism of the *SREBP1* gene in cattle was first reported by Hoashi et al. (2007) in Japanese Black and Holstein-Friesian cattle. They found no polymorphisms in the full-length coding sequence (CDS) region. However, they found the 84-bp indel polymorphism in intron 5 of the SREBP1 gene (84-indel) in Japanese Black cattle with two allele types. The 84-bp insertion is denoted as a long-type (or L allele), while the 84-bp deletion as a short-type (or S allele). These studies were then expanded to other cattle breeds.

SREBP1 or also known as sterol regulatory element binding transcription factor 1 (SREBF1) is also essential in the fat metabolism of ruminants because it also influences the fat characteristics of both meat and dairy products. This has been demonstrated in cattle (Harvatine & Bauman 2006; Proskura et al. 2017; Gamarra et al. 2021; Kawaguchi et al. 2021), buffalo (Deng et al. 2017; Xu et al. 2019), goat (Xu et al. 2016), and sheep (Tsiplakou et al. 2015; Liang et al. 2020; Carcangiu et al. 2021). In bovine, the *SREBP1* gene is located on chromosome 19 with 21 exons and encodes 1183 amino acids (Hoashi et al. 2007). The sequence of bovine SREBP1 mRNA has a high similarity (82%) to the SREBP1c isoform of hominids (Human and chimpanzee) (Gamarra et al. 2021). In addition, SREBP1 expression was positively correlated with FA in several cattle breeds (Gamarra et al. 2018). The differential mRNA expression of *SREBP1* has been reported in different tissues (Bhuiyan et al. 2009; Li et al. 2018) and different cattle breeds (Gamarra et al. 2018). The increased expression of *SREBP1* indicates an elevated capacity of *de novo* synthesis of FA, which leads to an enhanced lipid accumulation in bovine muscle (Li et al. 2018). *In vitro* studies in bovine hepatocytes and mammary epithelial cells showed that silencing or knocking down the SREBP1c gene decreased lipogenic gene expression and caused a decrease in lipid or FA synthesis (Ma & Corl 2012; Deng et al. 2014). In addition, lipopolysaccharides (LPS) from bacterial endotoxin can decrease milk fat synthesis via decreasing expression of SREBP1 in dairy cows (Wang et al. 2018). Therefore, the *SREBP1* gene is one of the promising candidate genes that determine FA composition and fat-related traits both in the meat of beef cattle (Han et al. 2013; Xu et al. 2013) and milk in dairy cattle (Harvatine & Bauman 2006; Nafikov et al. 2013; Li et al. 2014). Furthermore, SREBP1 together with other key FA metabolism-associated genes can be used for the genetic selection of healthy meat in beef cattle (Zhou et al. 2022).

Several studies have found several variants of the *SREBP1* gene that are spread in both coding and non-coding regions (Hoashi et al. 2007; Huang et al. 2011; Rincon et al. 2012; Lee et al. 2013). An 84-bp indel polymorphism (84-bp indel) in intron 5 of the *bovine SREBP1* gene was first reported by Hoashi et al. (2007). This polymorphism is popular and frequently found to affect the FA composition in several cattle breeds: Japanese Black cattle (Hoashi et al. 2007; Ohsaki et al. 2009), Hanwoo (Bhuiyan et al. 2009), Simmental (Xu et al. 2013), and Angus × Charolais crossbred (Han et al. 2013). The S allele (84-bp deletion) of the *SREBP1* gene has been found to contribute to elevated levels of good fatty acid than the L allele, and vice versa. Despite this, the polymorphism has also been found to affect carcass weight (Ohsaki et al. 2009) and growth traits in beef cattle (Huang et al. 2011)

Determinations of the genetic diversity of the 84-bp indel of the *SREBP1* gene among various cattle breeds in Indonesia is important to obtain the genetic marker for meat quality, especially for FA composition. However, information regarding the genetic diversity of the *SREBP1* gene in Indonesian cattle is limited. Therefore, the purpose of this study was to detect the genetic polymorphism of the 84-bp indel of the *SREBP1* gene in several cattle breeds in Indonesia i.e., Limousin, Simmental, Holstein-Friesian, Bali, Sumbawa, and Pasundan cattle breeds.

MATERIALS AND METHODS

DNA samples

This study involved a total of 740 animals, where 202 out of the samples of them were in the form of frozen semen collected from bulls at the two National Artificial Insemination Centers (NAICs) of Indonesia, i.e. Lembang and Singosari (consisting of 100 Limousin, 62 Simmental, and 40 Holstein-Friesian). An amount of 538 samples were collected from the blood of heifers and bulls kept by small-holder farmers (consisting of 100 Holstein-Friesian, 100 Sumbawa, 180 Bali, and 158 Pasundan). The sampling locations are presented in Table 1. Genomic DNA was extracted from both frozen semen and whole-blood samples by using a gSYNC DNA extraction kit (Genaid Biotech

Table 1. Sampling location for blood samples of cattle kept by small-holder farmers

Breed	Sampling Location (Districts)	n
Holstein-Friesian	Bogor, Enrekang, Sukabumi, Tasikmalaya	100
Sumbawa	Sumbawa	100
Bali	Barru, Enrekang, Klungkung	180
Pasundan	Ciamis, Majalengka, Pangandaran, Tasikmalaya	158

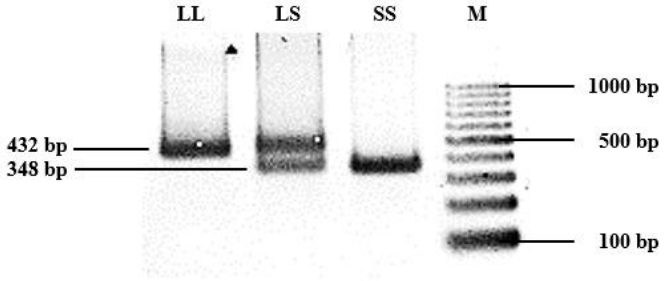

Figure 1. Visualization of the 84-bp indel polymorphism amplification products of the *SREBP1* gene in a 2% agarose gel (w/v). M: 100-bp DNA ladder. It showed genotypes of LL (432 bp), SS (348 bp), and LS (432 bp and 348 bp)

Ltd., Taiwan) and a blood/cell DNA mini kit (Genaid Biotech Ltd., Taiwan), respectively. All collected genomic DNA products were stored at -20 °C until further analysis was performed.

Genotyping

Genotyping of the 84-bp indel polymorphism in intron 5 of the bovine *SREBP1* gene (84-bp indel) was performed using the PCR amplification method. The primer pairs are according to Hoashi et al. (2007) with modification as follows: SREBP1-F: 5'-CCA CAA CGC CAT CGA GAA ACG CTA C -3' and SREBP1-R: 5'-GGC CTT CCC TGA CCA CCC AAC TTA G -3'. The PCR reactions were carried out in a total volume of 10 µL containing 10-12 ng/µL of DNA template, 4 µL of Go Taq Green Mastermix (Promega, USA), 0.2 µM of each primer (forward and reverse), and nuclease-free water up to a final volume of 10 µL. The PCR reaction was accomplished in a gradient thermal cycler (Eppendorf, Germany) under the following cycling conditions: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 95°C for 25 s, primer annealing at 59°C for 25 s, extension at 72°C for 25 s and final extension at 72°C for 5 min. The PCR products were electrophoresed in 2% of agarose gel at 100 V for 30 min and stained with GelRed®10,000X solution (Biotium, USA). The amplicons were visualized with a G-BOX Gel Documentation System (Syngene, UK).

The genotype of each individual was determined based on the pattern and size of the fragments observed in the gel. The 100-bp DNA ladder will be used as the standard for determining fragment size estimations. The LL genotype was indicated by the presence of one 432 bp fragment. The SS genotype is indicated by the presence of one 348 bp fragment due to an 84 bp deletion. Meanwhile, the LS genotype was shown with two fragments of 432 and 348 bp respectively. The representation of the fragment patterns of each genotype is shown in Figure 1.

Sequencing

The presence of 84-bp indel polymorphism was confirmed by forward and reverse sequencing reactions from one representative sample of different genotypes (LL, LS, and SS). The amplified products were sent to 1st BASE Sanger Sequencing services (Apical Scientific Sdn Bhd., Malaysia) for sequencing using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and capillary electrophoresis in ABI PRISM®310 Genetic Analyzer (Applied Biosystems, USA). The sequence data of the *SREBP1* gene in this study were then aligned with NCBI genome reference of NC_037346.1 and Genbank Accession number of AB355704.1 (as a representation of 84-bp insertion type) and AB355705.1 (as a representation of 84-bp deletion type) using the BioEdit software version 7.2 (Hall 1999).

Data analysis

Genetic diversity parameters were genotype frequencies (LL, LS, and SS), allele frequencies (L and

S), Hardy-Weinberg Equilibrium test, observed heterozygosity (Ho), and expected heterozygosity (He). All parameters were performed using GenAlEx v6.5 (Peakall & Smouse 2012).

RESULTS AND DISCUSSION

In the present study, specific DNA fragments of the bovine *SREBP1* gene were successfully amplified by producing three genotypes: LL (432 bp), LS (432 and 348 bp), and SS (348 bp) (Figure 1). An 84-bp indel polymorphism was also confirmed in this study by DNA sequence analysis (Figure 2). This polymorphism could be traced with rsID number rs133958066 at dbSNP database, which is relatively located between 34643011 to 34643094 (g.34643012_34643095del or c.1065+85_1065+168del) based on the bovine genome assembly NC_037346.1 (transcript ID: NM_0011113302.1) from the NCBI database or ENSBTAG00000007884 (transcript ID: ENSBTAT000) from the Ensembl database. This is equivalent to a base position between 84 to 167 under Genbank Accession No. AB355704.1 (AB355704.1:g.85_167del).

The genotype and allele frequencies of the 84-bp indel polymorphism are displayed in Table 2. In this study, the L allele was found to be dominant in all cattle breeds and was fixed in Holstein-Friesian (H-F), Bali, and Sumbawa cattle. Conversely, the S allele, as a minor allele, was detected in Limousin, Simmental, and Pasundan cattle with frequencies of 0.24, 0.16, and 0.01, respectively. As stated by Allendorf and Luikart (2007), a locus is considered polymorphic if the most common allele is at a frequency less than 99% (or 0.99). This suggests that the 84-bp indel locus was found to be polymorphic in Limousin, Simmental, and Pasundan cattle, while it was found to be monomorphic in Holstein-Friesian, Bali, and Sumbawa cattle. Additionally, while the frequency of homozygous SS genotypes was low in all breeds studied, Limousin cattle had the highest frequency of SS genotypes (0.07).

The previous studies on the genotype and allele frequencies of the 84-bp indel polymorphism in various cattle breeds are presented in Table 3. Our study supports previous findings, which showed the fixation of the L allele in H-F cattle (0.00) (Hoashi et al. 2007; Kaneda et al. 2011; Proskura 2013) indicating that the S allele was not present in this breed. On the other hand, Japanese Black cattle showed the highest frequency of the S allele among the breeds studied. The high frequency of the S allele (0.21) in Snow Dragon beef cattle is believed to originate mainly from Wagyu cattle, as Snow Dragon beef cattle are the result of crossbreeding between Wagyu (as a terminal-paternal breed) and F1-crossbred cows (Limousin x Fuzhou yellow cattle) (Liu et al. 2012). The frequencies of the S allele in Limousin (0.24) and Simmental (0.16) cattle in our study are consistent with previous studies on Limousin (0.22) (Bhuiyan et al. 2009) and Simmental (0.17) (Xu et al. 2013) cattle, respectively. However, Bhuiyan et al. (2009), did not detect the S allele in Simmental cattle (0.00) which might be due to the smaller sample size (n=49) compared to the study conducted by Xu et al. (2013) (n=314). As stated by Hartl (1988), the allele frequency within a given sample is an estimate of the true population allele frequency, and therefore, the larger the sample size, the more accurate the estimate. Based on this, Hartl (1988) recommended using a sample of 100 individuals or more whenever possible. Meanwhile, the S allele was at a very low frequency in Pasundan cattle (0.01). This has also been observed in Brahman (0.05) and Red Chittagong (0.01) (Bhuiyan et al. 2009), as well as Canadian crossbreeds (0.01) (Han et al. 2013).

Table 2. Genotype and allele frequencies of the 84-bp indel polymorphism in the *SREBP1* gene in various cattle breeds in Indonesia

Breed	N	Genotype frequency			Allele Frequency		$\chi 2$	Prob	Sig	Ho	He
		LL (n)	LS (n)	SS (n)	L	S					
Limousin	100	0.58 (58)	0.35 (35)	0.07 (7)	0.76	0.24	0.291	0.590*	ns	0.350	0.370
Simmental	62	0.69 (43)	0.29 (18)	0.02 (1)	0.84	0.16	0.331	0.565*	ns	0.290	0.271
Holstein-Friesian	140	1.00 (140)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	-	-	-
Bali	180	1.00 (100)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	-	-	-
Sumbawa	100	1.00 (100)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	-	-	-
Pasundan	158	0.97 (154)	0.03 (4)	0.00 (0)	0.99	0.01	0.026	0.872*	ns	0.025	0.025

N= number of individual samples, χ^2= Chi-squared value, prob= probability, ns = not significant. *The probability of the observed chi-squared value being greater than 0.05 (P>0.05) means that the population is in Hardy-Weinberg Equilibrium, H = Observed heterozygosity, He= Expected heterozygosity.

Table **3**. Genotype and allele frequencies of the 84-bp indel polymorphism in the *SREBP1* gene in various cattle breeds reported in previous studies

Breed	N	Genotype frequency			Allele Frequency		Ho*	He*	Ref
		LL (n)	LS (n)	SS (n)	L	S			
Japanese Black[1]	72	0.39 (28)	0.49 (35)	0.12 (9)	0.63	0.37	0.49	0.46	Hoashi et al. (2007)
Japanese Black[2]	606	0.16 (98)	0.72 (437)	0.12 (71)	0.52	0.48	0.72	0.50	Hoashi et al. (2007)
Japanese Black[3]	417	0.46 (190)	0.47 (196)	0.07 (31)	0.69	0.31	0.47	0.43	Ohsaki et al. (2009)
Japanese Black[4]	233	0.45 (106)	0.43 (101)	0.11 (26)	0.67	0.33	0.43	0.44	Ohsaki et al. (2009)
Japanese Black[5]	480	0.24 (115)	0.49 (235)	0.27 (130)	0.49	0.51	0.49	0.50	Matsuhashi et al. (2011)
Japanese Black[6]	539	0.47 (256)	0.41 (219)	0.12 (64)	0.68	0.32	0.44	0.44	Sasazaki (2021)
Holstein-Friesian	96	1.00 (96)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Hoashi et al. (2007)
Holstein-Friesian	30	1.00 (30)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Kaneda et al. (2011)
Holstein-Friesian	175	1.00 (175)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Chinese Holstein	94	1.00 (94)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Huang et al. (2011)
Bos indicus[7]	30	1.00 (30)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Kaneda et al. (2011)
Jersey	50	1.00 (50)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Charolais	43	1.00 (43)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Angus	49	1.00 (49)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Montbéliarde	187	0.55 (103)	0.41 (77)	0.04 (7)	0.76	0.24	0.41	0.37	Proskura (2013)
Nanyang	265	0.85 (226)	0.15 (39)	0.00 (0)	0.93	0.07	0.15	0.14	Huang et al. (2011)
Qinchuan	235	0.90 (211)	0.10 (24)	0.00 (0)	0.95	0.05	0.10	0.10	Huang et al. (2011)
Jiaxian Red	441	0.73 (322)	0.27 (119)	0.00 (0)	0.87	0.13	0.27	0.23	Huang et al. (2011)
Hanwoo	62	0.52 (46)	0.40 (35)	0.08 (7)	0.72	0.28	0.40	0.40	Bhuiyan et al. (2009)
Hanwoo	348	0.59 (206)	0.30 (104)	0.11 (38)	0.74	0.26	0.30	0.38	Lee et al. (2013)
Canadian crossbred[8]	225	0.98 (220)	0.02 (5)	0.00 (0)	0.99	0.01	0.02	0.02	Han et al. (2013)
Simmental (bulls)	314	0.67 (212)	0.32 (99)	0.01 (3)	0.83	0.17	0.31	0.28	Xu et al. (2013)
Snow Dragon Black	128	0.62 (79)	0.34 (44)	0.04 (5)	0.79	0.21	0.34	0.33	Xu et al. (2013)

N= number of individual samples; [1]Collected from all over regions in Japan; [2]Collected from progeny testing station at Hokkaido and Hiroshima; Japan, [3]Collected from field populations in Miyazaki, Japan; [4]Collected from all over Japan for field progeny testing carried out by the Wagyu Registry Association; [5]Collected from commercial populations in Gifu, Japan; [6]Collected from Hyogo Prefecture, Japan; [7]Consisted of 10 Cambodian, 10 Myanmar, and 10 Laotian native cattle; [8]Crossbred (Angus x Charolais) commercial steers; *Calculated from genotypic and allelic data in cited references

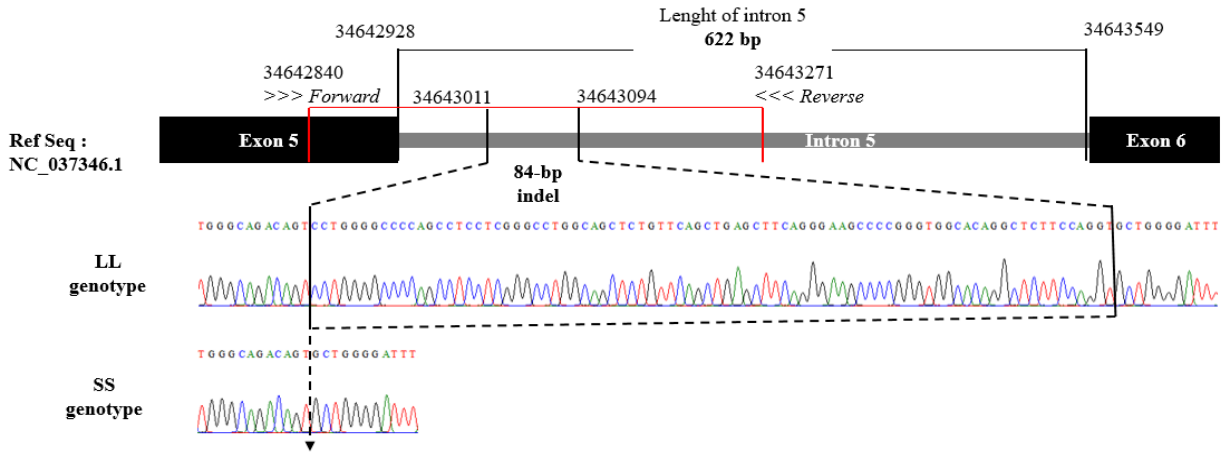

Figure 2. Chromatogram sequence comparison between long-type (LL genotype) and short-type (SS genotype) sequences of 84-bp indel polymorphism in intron 5 of the bovine *SREBP1* gene. Redline is the position of flanking primers (forward and reverse) used in the present study. The chromatogram showed the absence of an 84-bp sequence in the SS genotype.

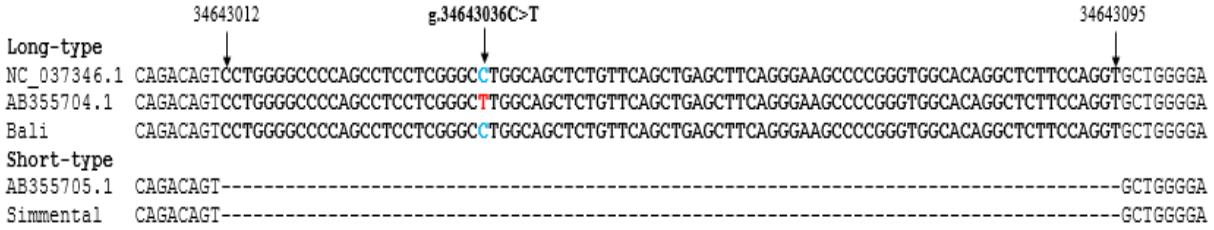

Figure 3. Sequence alignment of long-type and short-type of 84-bp indel polymorphism of the bovine *SREBP1* gene between Genbank (NC_037346.1, AB355704.1, and AB355705.1) and representative sample sequences in the present study (Bali and Simmental). The 84-bp insertion sequences are indicated in black bold letters. Novel SNP is indicated in blue and red letters.

The observed heterozygosity (Ho) values for Limousin, Simmental, and Pasundan cattle were 0.35, 0.29, and 0.02, respectively. This suggests that the level of heterozygosity was low in Pasundan cattle, while Limousin and Simmental cattle had a higher Ho value compared to Nanyang (0.15), Qincuan (0.10), and Canadian crossbred cattle (0.02). On the other hand, Japanese Black cattle had the highest Ho value ranging from 0.43 to 0.72 among all the cattle breeds studied which ranged from 0.02 to 0.41. Therefore, the 84-bp indel marker has a high potential to be used in marker-assisted selection in Japanese Black cattle.

The frequency of the L allele was observed to be higher than that of the S allele in all cattle breeds studied. Gamarra et al. (2021), speculate that the presence of the S allele in some *Bos taurus* breeds could have appeared after *Bos indicus* and *Bos taurus* species differentiation around 1.7 to 2 million years ago. The S allele is thought to be specific and may not be segregating widely among different cattle breed populations as has been declared in several previous reports (Hoashi et al. 2007; Bhuiyan et al. 2009). To enrich the information on S and L allele frequencies in various breeds, we also analyzed the genotypes of other bull breeds in NAICs, including Angus (n=9), Brangus (n=9), Ongole (n=22), Brahman (n=6), Aceh (n=4), and Madura (n=5). The results indicated that the S allele was not found in all of the bulls (unpublished data). Therefore the S allele tends to be absent in *Bos indicus* breeds as observed in Cambodian, Myanmar, and Laotian native cattle (Kaneda et al. 2011) as well as in Sumbawa cattle. In addition, our study provides new information that the S allele was also absent in the *Bos javanicus* species (Bali cattle). Meanwhile, the presence of a small number of individuals carrying the S allele in Pasundan cattle (*Bos indicus* x *Bos javanicus*) could not be explained with certainty whether the S allele was segregated in the population or due to crossbreeding with *Bos taurus* cattle as presumed in *F94L-MSTN* mutation (Anwar et al. 2020).

The SREBP family of transcription factors plays a crucial role in regulating the transcriptional activation of genes involved in the synthesis of fatty acids, particularly those that contain the sterol regulatory element (SRE). SREBPs bind to SREs located in the promoter regions of target genes, thus affecting gene expression. Target genes of SREBP1c isoform as in bovine, including low-density lipoprotein (LDL) receptor, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), insulin-induced gene-1 (INSIG1), S14, glucokinase (GK), phosphoenolpyruvate carboxykinase (PEPCK),

and sodium/iodide symporter (NIS) which are important in milk fat synthesis, glucose synthesis and lactation in cattle (Rincon et al. 2012; Li et al. 2014; Wen et al. 2016). Target gene activation by SREBP has been demonstrated in several studies. The study conducted by Wen et al. (2016) proved that there was a decrease and increase in mRNA expression and protein concentration of NIS through inhibition, silencing, and overexpression of SREBP and by reporter gene and DNA-binding assay experiments. A study by Gamarra et al. (2018) showed a significant positive correlation between SREBP1 and SCD1 gene expression levels in several cattle breeds. The important role of SREBP was also shown in the study of Han et al. (2018) where the promoter activity of FASN2 and FASN3 was due to overexpression of SREBP1. However, this effect can be enhanced by the co-overexpression of SCAP + SREBP1, where SCAP is an essential protein for SREBP activation. However, the 84-bp indel polymorphism of SREBP1 itself was found to not affect SCD1 gene expression. Furthermore, Hoashi et al. (2007) found no interaction between one SNP in the SCD1 gene and the 84-bp indel polymorphism of the SREBP1 gene in influencing the percentage of monounsaturated fatty acids (MUFA) and melting points in intramuscular fat. This suggests that the 84-bp indel might influence the transcription efficiency of SREBP1 itself and thus have an indirect effect on the quality of fat in cattle.

Although it is unclear the effect of the presence of the 84-bp indel in intron 5 of the SREBP1 gene on gene regulation, the association between 84-bp indel polymorphism and FA composition in several beef cattle breeds has been reported in several previous studies. In general, the S allele was favored as it is significantly associated with increased levels of healthy FAs (Hoashi et al. 2007; Bhuiyan et al. 2009; Gamarra et al. 2021). Even though some studies found no significant effect in commercial Japanese Black, and commercial Korean cattle (Matsuhashi et al. 2011; Lee et al. 2013) and inconsistent results between progeny testing and field populations in Japanese black cattle (Ohsaki et al. 2009). This could be due to the relatively small effect of the *SREBP1* gene on the field population (Ohsaki et al. 2009) or may have a different linkage phase between the DNA marker and the causative mutations in different beef cattle populations (Han et al. 2013).

In addition to FA compositions, the 84-bp indel was also associated with growth and carcass traits and body size in some cattle breeds. Ohsaki et al. (2009) found that the SS genotype has a higher carcass weight ($P = 0.0451$) than LS and LL genotypes in Japanese Black cattle. Huang et al. (2011), reported that the LS genotype had significantly higher birth weight, body weight, and average daily gain compared to the LL genotype at birth, 6-, 18-, and 24-month old ($P < 0.05$ or $P<0.01$) in Nanyang cattle. A recent study showed that *SREBP1* gene 84-bp indel was significantly associated with back fat thickness, ultrasound *longissimus muscle* depth, and body size traits in Chinese Qincuan cattle (Gao et al. 2022). In contrast, in the case of Xinjiang brown dairy cattle, Peng et al. (2020) found that 84-bp indel was significantly associated with 305-day milk production and milk protein yield in the second and third parity, but the SS genotype tended to be lower than the LS and LL genotypes.

Introns are non-coding sequences and therefore variations in the intronic regions are considered to have less functional significance compared to variations in the exonic regions in determining specific traits. Despite this, some studies have demonstrated an association between variants in the intronic regions of certain genes and the fatty acid composition of both types of meat (Srikanth et al. 2015; Otto et al. 2022) and milk (Jiang et al. 2016; Li et al. 2016) in cattle. According to Hoashi et al. (2007), the 84-bp indel polymorphism in the intronic region of the SREBP1 gene can directly affect the translation efficiency of the gene, and the presence of insertion of 84 nucleotides is thought to have a regulatory-like function on micro RNA (miRNA) in controlling the expression of genes related to fat quality in cattle. However, this argument needs to be further investigated.

Micro RNA (miRNA) is a small non-coding RNA (ncRNA) that regulates gene expression (Catalanotto et al. 2016). It works by binding to specific regions within its target mRNA, such as the promoter, 5'UTR, 3'UTR, or coding sequences, to either inhibit, silence or induce the expression of target genes (O'Brien et al. 2018). Although the mechanism of the 84-bp indel in the intron 5 of the *SREBP1* gene in influencing fat quality has not been reported, several studies have shown the effect of indel polymorphisms in the intronic region on the expression of genes and/or phenotype in various animals such as chicken (Zhang et al. 2014), pig (Cui et al. 2019), goat (Gao et al. 2020; Wang et al. 2020), and cattle (Zhao et al. 2018; Jiang et al. 2019). Cui et al. (2019) reported that three intronic indels (11-bp and 16-bp insertions and 17-deletion) are associated with the mRNA expression of the KDM6A gene and testicular measurement traits in male piglets. In addition, the *in silico* analysis showed that the presence of the indel in the KDM6A gene affects the binding ability of several transcription factors in transcription binding sites.

Interestingly, according to the results of the sequence alignment analysis in our study, there is evidence of an SNP within an 84-bp insertion-type sequence at the position 34643036 according to RefSeq NC_037346.1 (g.34643036C>T). The sequence in RefSeq NC_037346.1 and Bali cattle was found to be Cytosine (C), while it was Thymine (T) in Japanese

Black cattle (Genbank: AB355704.1), as shown in Figure 3. This SNP may be a breed-specific variant exclusive to Japanese cattle populations. However, further confirmation is necessary through studies on larger cattle breed populations.

CONCLUSION

This study concluded that the L allele (insertion-type) was found to be a common allele across all breeds studied. Furthermore, the 84-bp indel of the *SREBP1* gene was found to be polymorphic only in Limousin, Simmental, and Pasundan cattle, but monomorphic in Holstein-Friesian, Bali, and Sumbawa cattle.

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Changes in the Qualitative Composition of the milk of Holstein Cows During Summer Chronic Heat Stress

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ABSTRAK

Mylostyvyi R, Midyk S, Izhboldina O, Cherniy N, Kornienko V. 2023. Perubahan komposisi kualitatif susu sapi Holstein selama stress musim panas. JITV 28(2):112-121. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3151.

Stres musim panas mengakibatkan kerugian ekonomi yang signifikan dengan menurunnya produksi dan kualitas susu sapi perah. Tujuan dari penelitian ini adalah untuk mengetahui perubahan parameter kualitatif susu sapi Holstein selama mengalami stres panas kronis di salah satu peternakan sapi perah komersial terbesar di Ukraina (50°49′14″ N, 31°49′23″ E). Lima ekor sapi multipara laktasi yang memproduksi susu sekitar 30 kg per hari dipilih secara acak menjadi kontrol (pada musim semi, akhir Mei) dan kelompok eksperimen (pada musim panas, pada akhir Agustus). Sampel susu diambil pada saat pemerahan pagi hari. Analisis kualitatif susu meliputi identifikasi susu padat-tidak-lemak, densitas, fraksi massa laktosa, lemak, protein dan mineral, serta titik beku, daya hantar listrik dan keasaman aktif dengan metode ultrasonik. Ternak dipelihara di kandang berventilasi alami. Pakan campuran seimbang tipe-tunggal terdiri dari silase jagung dan konsentrat yang diterima sapi tetap tidak berubah. Pengambilan sampel susu dari sapi kelompok eksperimen dilakukan selama 26 hari musim panas secara terus-menerus. Nilai indeks suhu dan kelembaban harian maksimum tidak turun di bawah 72 unit. Hasil penelitian menunjukkan bahwa terjadi penurunan kandungan padat-tidak-lemak, fraksi massa lemak dan protein ($P<0,05$) pada susu hewan eksperimen. Fraksi massa laktosa dan mineral cenderung meningkat. Perlu dicatat bahwa rata-rata produksi susu harian sapi perah dalam kawanan di musim semi dan musim panas hampir sama. Kesimpulannya, hasil penelitian menunjukkan bahwa suhu yang diproduksi pada musim panas ekstrim menyebabkan penurunan kualitas susu sapi Holstein. Meskipun kepadatan susu dan titik beku menurun, angka-angka ini memenuhi persyaratan standar. Indikator organoleptik susu, konduktivitas listrik dan keasaman aktif susu tidak berubah dalam cuaca panas, nilainya menunjukkan kealamian susu. Fraksi massa lemak susu, yang mengalami perubahan terbesar di bawah pengaruh tekanan panas musiman, merupakan salah satu komponen susu yang paling berharga, yang berdampak langsung pada nilai gizi dan harga pembelian susu mentah. Oleh karena itu, studi lebih lanjut tentang komposisi asam lemak susu menggunakan metode kromato-spektrometri massa akan memberikan data berharga yang diperlukan untuk mencari kemungkinan strategi pengelolaan ternak untuk mempertahankan kualitas susu yang tinggi dalam kondisi cekaman panas musiman.

Key Words: Sapi Perah, Indikator Organoleptik, Kandungan Fisika Kimia Susu, Cuaca Panas Berkepanjangan

ABSTRACT

Mylostyvyi R, Midyk S, Izhboldina O, Cherniy N, Kornienko V. 2023. Changes in the qualitative composition of the milk of Holstein cows during summer chronic heat stress. JITV 28(2):112-121. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3151.

Seasonal summer heat stress leads to significant economic losses, resulting in a drop in milk yield in dairy cows and a deterioration in milk quality. The purpose of this study was to determine the changes in some qualitative parameters of milk in Holstein cows during chronic heat stress on one of the largest commercial dairy farms Ukraine (50°49′14″ N, 31°49′23″ E). Five multiparous cows of medium lactation with a milk yield of about 30 kg per day were randomly selected into the reference (in the spring, at the end of May) and the experimental group (in the summer, at the end of August). Milk samples were taken from cows during the morning milking. Qualitative analysis of milk included the identification of milk solids-not-fat, density, mass fraction of lactose, fat, protein and minerals, as well as the freezing point, electrical conductivity and active acidity using ultrasonic method. Animals were kept in naturally ventilated barns. The total mixed single-type balanced diet consisting of corn silage and concentrates that the cows received remained unchanged. The sampling of milk from cows of the experimental group was preceded by a continuous 26-day hot period, during which the maximum daily values of the temperature and humidity index did not fall below 72 units. The results showed that in the milk of the animals of the experimental group there was a significant decrease in the content of milk solids-not-fat, the mass fraction of fat and protein ($P<0.05$). The mass fraction of lactose and minerals tended to increase. It should be noted that the average daily milk yield of dairy cows in the herd in spring and summer was almost at the same level. In conclusion, the results of the study showed that high summer temperatures lead to a deterioration in the quality of milk in Holstein cows. Despite the decrease in milk density and freezing point, these figures met the requirements of the standard.

Organoleptic indicators of milk, electrical conductivity and active acidity of milk did not change in hot weather, their value indicated the naturalness of milk. The mass fraction of milk fat, which undergoes the greatest change under the influence of seasonal heat stress, is one of the most valuable components of milk, which has a direct effect on nutritional value and purchase price of raw milk. Therefore, a further deeper study of the fatty acid composition of milk using the method of chromato-mass spectrometry will provide valuable data necessary to search for possible herd management strategies to maintain high milk quality under conditions of seasonal heat stress.

Key Words: Dairy Cows, Organoleptic Indicators, Physico-Chemical Properties of Milk, Prolonged Hot Weather

INTRODUCTION

One of the gravest environmental problems facing humanity is global climate change. According to the forecasts of the leading world climate research centers, air temperature on the globe will be increasing by 2-5 degrees Celsius over the next century (National Ecological Center of Ukraine). Such a rate of global warming can cause severe climate change, so some plant and animal species will be in danger of extinction. It may well be argued that major climate change is happening now, which is a challenge for global agriculture.

Agriculture in general and livestock breeding in particular are primarily and most sensitive to all environmental changes (Abdela & Jilo 2016). Therefore, the sustainable development of livestock breeding remains an urgent problem under global climate change. It is with increasing annual temperatures and possible heat stress that most scientists associate the negative effects of these processes on dairy cattle raising (Herbut et al. 2018). According to Escarcha et al. (2018), short-term extreme weather events will have the greatest impact on feed production, animal health and product quality, and therefore food security is at threat today in many parts of the world, which needs to be addressed in long-term strategies for livestock adaptation to climate change (Wangui et al. 2018).

Despite some success in mitigating the heat stress impact on dairy farming, there has been a noticeable downward trend in production during hot periods in summer (Dunshea et al. 2013). Major losses in the dairy industry due to the manifestation of seasonal heat stress are associated with a decrease in cows' milk yields and changes in milk components that lead to a dairy products deterioration (Maggiolino et al. 2020).

Heat stress not only reduces milk production, but also affects somatic cell count and milk composition, which are particularly susceptible to change even with slight increases in ambient temperatures beyond the physiological comfort of cows (Nasr MAF & El-Tarabany 2017; Bertocchi et al. 2020). For example, the fat and protein content of milk reduced by 0.012 kg and 0.009 kg, respectively, as a result of an increase in temperature and humidity index (THI) per unit increase in THI above 72 (Liu et al. 2019). A little earlier it was reported that when the average THI increased from 68 to 78 (from spring to summer), the protein and fat content of milk reduced from 2.96% and 3.58% to 2.88% and 3.24%, respectively, with a drop in milk yield by 21% (Herbut & Angrecka 2012). Studies by Maggiolino et al (2020) confirm that the above changes in the composition of milk under heat stress have a negative impact on the technological properties of milk and especially on cheese production. The objective of this study was to determine the changes in some qualitative parameters of milk in Holstein cows during chronic heat stress.

MATERIALS AND METHODS

Animals involved in the experiment

This experiment was conducted in accordance with the animal welfare requirements and approved by the Bioethics Commission of the Dnipro State Agrarian and Economic University. Ten multipara milking Holstein cows (160±14 days of lactation) were randomly assigned to one of the two groups. Some of the cows were assigned to the control group (n=5) in May (spring season) and the other part was referred to the experimental group (n=5) in August (summer). The average daily milk yield in cows of the control group was 30.4±0.17 kg/day, in cows of the experimental group - 31.0±0.26 kg/day, there was no significant difference in milk yield.

Weather conditions

Weather data from the nearest weather station was taken on the website of the Ukrainian Hydrometeorological Center, as described earlier (Mylostyvyi & Chernenko 2019). The data (temperature and relative humidity) was recorded every three hours per day. At the same time, the temperature-humidity index (THI), calculated according to Kibler (1964), was used as the indicator of heat stress (HS) in cows.

$$THI = 1.8 \times T - (1 - RH/100) \times (T-14.3) + 32$$

where THI is the temperature-humidity index, T is the air temperature in °C, and RH is the relative humidity in %.

The THI limit, at which most dairy cows could experience HS was considered to be 72 units (Herbut et al. 2018). Weather data was taken into account within 24 hours during the studies, as well as during the week preceding the studies.

Maintenance, feeding and milking of cows

The research was carried out on one of the large commercial dairy farms in Ukraine (50°49′14″ N, 31°49′23″ E). In short, milking Holstein cows were kept unattached in a naturally ventilated barn (NVB). Rubber mats were used as bedding in the stalls. The cows had a general mixed diet including the own-produced fodder, which consisted of corn silage, lucerne haylage, cereal hay, wheat straw, barley grain, oats and corn. Rapeseed, sunflower and soybean meal, dried pulp, as well as mineral and vitamin supplements were also included in the diet. The fed rations were balanced in terms of essential nutrients according to the recommendations of the National Research Council (NRC 2001). The premises had a feeding table and freely accessible group drinking bowls. The fed ration was not changed during

the year. The animals were kept permanently indoors, with no grazing. DeLaval equipment, a Cascade milking room for 72 cows with a production capacity of 300 heads per hour and computer identification of animals in the DairyComp 305 herd management system were used for milking cows. The milk is completely isolated from the external environment, which guarantees its high sanitary performance. The milking of cows is performed three times a day in all seasons.

Research facilities and indicators measured

The milk was studied in the Ukrainian Laboratory of Quality and Safety of Agricultural Products of NUBiP of Ukraine, accredited as per the quality system of DSTU EN ISO/IEC 17025:2019. Samples were taken from chilled bulk milk according to (DSTU ISO 707:2002 2002; DSTU 8553:2015 2015).

Organoleptic parameters of milk were identified: appearance, colour, taste, smell, and consistency. According to DSTU 3662:2018, which is in force in Ukraine, milk of all (three) grades must meet the following requirements in terms of organoleptic characteristics: consistency: homogeneous liquid without protein flakes and sediment; taste and smell: pure, typical of fresh milk, without foreign flavors and odors; color: from white to light cream.

According to DSTU 7057:2009, the density, mass fraction of fat, protein, and lactose were measured by the ultrasonic method on the Master Classis LM2PI milk analyzer. The same device, in addition, assessed: active acidity, dry skimmed milk residue, freezing point, electrical conductivity, mass fraction of minerals, mass fraction of water and freezing point.

Based on these indicators, according to DSTU 3662:2018, milk is divided into 3 grades: extra (density ≥1028.0 kg/m3, mass fraction of solids ≥12%; acidity: pH 6.6-6.7; freezing point (minus) ≥0.520°C), premium (density ≥1027.0 kg/m3, mass fraction of solids ≥11.8%; acidity: pH 6.6-6.7; freezing point (minus) ≥0.520°C), first grade (density ≥1027.0 kg/m3, mass fraction of solids ≥11.5%; acidity: pH 6.55-6.8; freezing point (minus) ≥0.520°C).

Statistical analysis

For statistical data processing, the statistical software package STATISTICA 12 (StatSoft, Inc., Tulsa, OK, USA) was used. The distribution of all the variation series was subject to the normality test. The data is represented as mean and standard error of mean (SE). The significance of the differences between the groups was assessed using Student's t-test. The difference with values of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Weather conditions during the studies

Milk sampling in the cows of the experimental group was preceded by a 26-day period of continuous heat stress for the milk cows (THImax ≥72). At the time of research, the maximum daily value of THI was about 75 units (Table 1).

Considering the existing close relationship between the environment and the body, the direct influence of meteorological factors on the physiological state of productive animals, the assessment of the animals' comfort using integral indicators or indices deserves special attention. The temperature-humidity index, which is based on air temperature and relative humidity measurements, has traditionally been used to quantify the degree of HS in animals. THI is easy to calculate and it is rather informative. In the reference group, milk sampling was carried out under thermal comfort (THI=67).

Table 1. Weather conditions on the day and during the week prior to milk sampling

Indicator	Reference Group			Experimental Group		
	Me	min	max	Me	min	max
	Weather conditions on the day of milk sampling					
Temperature, °C	17.5	14.0	21.0	22.6	19.0	29.0
Relative humidity, %	66.9	57.0	77.0	51.4	35.0	56.0
THI, units	62.4	57.3	67.1	68.6	64.2	74.8
	Weather conditions during the week prior to milk sampling					
Temperature, °C	19.0	14.0	25.0	22.7	16.0	32.0
Relative humidity, %	73.0	56.0	100.0	56.1	35.0	100.0
THI, units	63.1	55.7	71.6	69.0	60.4	78.5

Parameters of the external environment were recorded according to the data from the nearest weather station

Table 2. Compliance of milk with procurement requirements by organoleptic characteristics

Indicators	Reference Group	Experimental Group	Characteristics according to DSTU 3662
Consistence	MS	MS	homogeneous liquid with no protein floccules and sediment
Taste and smell	MS	MS	inherent in fresh milk, with no extraneous flavours and odours
Color	MS	MS	from white to light-cream and a slight yellowish shade

Meets the standard (MS)

Table 3. Results of measuring the quality of milk obtained under normal conditions and under chronic heat stress (Mean±SE, n=5)

Indicators, units of measurement	Reference Group	Experimental Group
Milk Solids-Not-Fat, %	8.69±0.04	8.09±0.08*
Mass fraction of fat, %	3.73±0.01	3.50±0.05*
Density, kg/m^3	1031±0.14	1029±0.14
Mass fraction of protein, %	3.24±0.02	3.03±0.06*
Mass fraction of lactose, %	5.16±0.02	5.34±0.08
Mass fraction of minerals, %	0.76±0.12	0.86±0.10
Mass fraction of water, %	0.00	0.00
Freezing point, (minus) ^{0}C	0.590±0.003	0.542±0.008
Electrical conductivity, mS/cm	5.6±0.01	5.6±0.03
Active acidity, pH units	6.63±0.02	6.63±0.03

*P<0.05

Organoleptic parameters of milk

In terms of organoleptic indicators, the milk of the reference and experimental groups corresponded to the current regulations of Ukraine (DSTU 3662:2018) and had the following characteristics (Table 2). Thus, the data show that the basic organoleptic characteristics of the milk of the control and experimental groups were unchanged and fully complied with the current regulatory document in Ukraine.

Milk solids-not-fat

The most variable proportion of milk solids is fat, therefore in dairy production, the indicator of the content of milk solids-not-fat (MSNF) is often used, the amount of which is obtained after subtracting the percentage of fat from the total amount of milk solids (Ilchuk et al. 2016).

This indicator is not standardized according to the regulatory documents of Ukraine, but in our studies we observed a probable MSNF reduction by 6.9% in the experimental group compared to the reference group (Table 3).

The amount of milk solids is associated with its chemical composition, in particular fat and protein content (Evers et al. 2021). The nature of seasonal changes in the content of milk solids is similar to the nature of seasonal changes in fat and protein: low content of milk solids is noted in the spring and summer period, while it is higher in autumn and winter. This is mainly traditionally associated with the changes in feeding and the consumption of juicy green fodder during grazing (Polieva et al. 2021). There is also additive genetic variability for productive traits in dairy cattle associated with the use of genetic breeding programs (Uribe & Gonzalez 2019).

But in our case, the diet of animals did not change. However, a decrease in the content of dry matter, milk fat and protein were observed. This can be explained by the direct effect of heat stress on the body of dairy cows. Since other researchers have also reported a decrease in these milk components under heat stress (Gao et al. 2017; Qin et al. 2018; Mavangira et al. 2018; Koshshavka et al. 2020).

Mass fraction of fat

The synthesis of lactic fat depends on fatty acids. Milk fat contains characteristic fatty acids. Under the influence of heat stress, significant changes in metabolic processes occur in the body of dairy cows (Mylostyvyi et al. 2021b), which also leads to changes in milk fatty acids (Penev et al. 2021). For example, the study of milk fat composition when cows are kept at 32. 2°C showed significant changes in the fatty acid composition of milk associated with a decrease in lauric (C12:0), myristoleic (C14:1), pentadecanoic (15:0), oleic (C18:1) and linoleic (C18:2) fatty acids with a simultaneous increase in palmitic (C16:0) and stearic (C18:0) fatty acids compared to cows under isothermal conditions (Moody et al. 1971).

Milking cows suffering from heat stress often show a decrease in milk fat content, which can cause losses to producers in the difficult conditions of the today's dairy market (Liu et al. 2019).

The fat content in the milk of the experimental group of cows is reduced by 6.2% relative to the reference group. Our results are consistent with those of other authors (Qin et al. 2018; Mavangira et al. 2018), reporting a decrease in lactic fat content in cows due to heat stress.

Mass fraction of protein

This is the main indicator that characterizes the naturalness of milk. The naturalness of milk is very important for the Ukrainian market of milk consumers, as people want to consume natural milk. The level of falsification of the Ukrainian dairy market exceeds 50% (Karpenko 2020). It is also a decisive factor on which the cheese-making properties of milk and the yield of cheeses depend. The mass fraction of protein in milk corresponded to the baseline in the reference group. However, under conditions of chronic heat stress, it tended to decrease by 6.5% relative to the protein content in the milk of cows under isothermal conditions (Figure 1).

Milk density

This indicator is widely used to convert the amount of milk expressed in kilograms into liters and vice versa; to establish and control the naturalness of milk; to calculate dry matters, milk solids-not-fat (MSNF) and other components using relevant formulae.

Density refers to the ratio of the mass of a substance to its volume. The milk density is represented by the ratio of the milk mass at a temperature of 20°C to the mass of an equal volume of water (t = 4°C). The density of whole cow's milk ranges from 1.027-1.033 (in individual animals from 1.026 to 1.031). These fluctuations depend significantly on the breed, feeding and housing conditions of the animals, mainly on the quantitative changes in the milk components, conditioning the milk density. The milk density in the milk of the experimental group of cows is reduced by 0.2 % compared to the reference group. In this study, the decrease in milk density is not an indication of milk falsification, but is a consequence of the effect of heat stress on the organism of dairy cows.

Mass fraction of lactose

Lactose in milk is the most stable component, the content of which hardly changes during lactation. This is a very important factor, since milk sugar plays a major role in maintaining a constant osmotic pressure in the

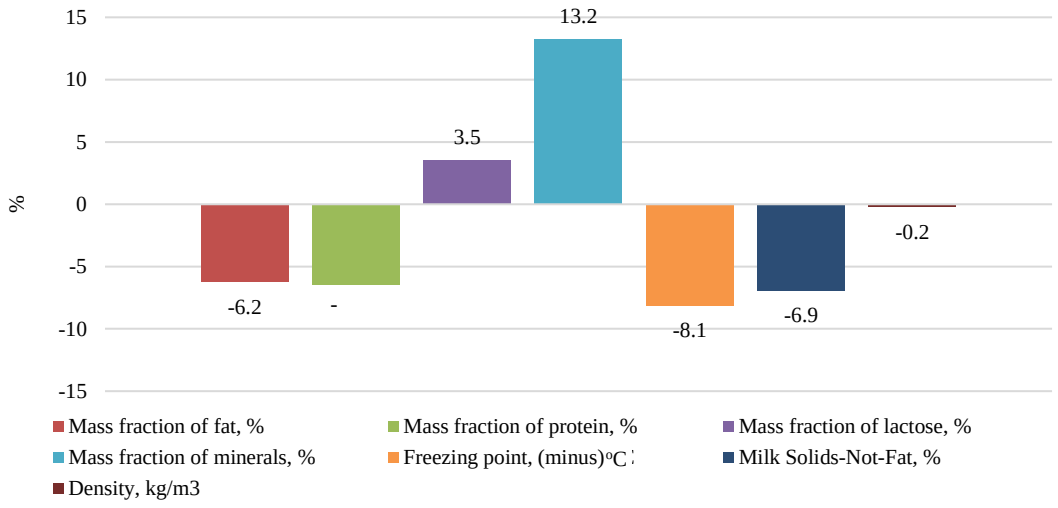

Figure 1. Changes in the quality indicators of raw milk (in %) with moderate heat stress in cows compared to milk obtained from cows of the reference group

blood-milk system (Antoniuk 2016). Lactose is an osmotically active substance that conditions the volume of secretion of water with milk and, accordingly, is the main factor contributing to the level of milk yield, due to which the fluctuation of its content in milk is much lower than that of fat and protein (Tian et al. 2016). In our studies, the lactose content in the experimental group increased by 3.5% compared to the control group. Other researchers have also reported changes in the lactose content of milk from Brazilian Holstein cows during heat stress (Garcia et al. 2015).

Mass fraction of minerals

Milk contains about 1% of minerals, which include 80 elements of the Mendeleev's periodic table. The salt of milk constitutes a small portion of milk (8-9 g.L(-1)); this component contains calcium, magnesium, sodium and potassium for the main cations and inorganic phosphate, citrate and chloride for the main anions. In milk, these ions are more or less associated between themselves and with proteins. Depending on the type of ion, they are diffusible (cases of sodium, potassium and chloride) or partially associated with casein molecules (cases of calcium, magnesium, phosphate and citrate), to form large colloidal particles called casein micelles (Gaucheron 2005).

This indicator is not standardized by the regulations of Ukraine. In our experiment, the mass fraction of minerals in the milk of the experimental group of animals tends to increase by 13.2% compared to the reference group.

Mass fraction of water and freezing point

The water in milk indicates its falsification or violation of the processing technology. Therefore, there must be no water in the raw milk. This metric is related to the freezing point.

When milk is frozen and crystallized, energy is released in the form of structural heat, measured (by measuring the temperature) using direct cryoscopic methods (Tsekhmistrenko & Kononskyi 2014). The freezing point value is due to the number of truly soluble components of milk (lactose and mineral salts), the content of which in milk does not significantly change. When water is added to milk, the concentration of water-soluble substances changes, resulting in a change in the freezing point of milk (Rusko 2011).

Milk freezing temperature (point) is also currently controlled by Regulation (EC) No. 853/2004 within the range of 0.515. In DSTU 3662:2018, this indicator is not lower than 0.520. In the cows of the experimental group, there was a lowered freezing point by 8.1% compared to the reference group, remaining within the acceptable range.

Conductivity

An important indicator of milk quality is electrical conductivity. The conductivity of milk at 20°C is 3-6 mS/cm and varies depending on the concentration of ions in it. The addition of salts increases the concentration of ions and therefore increases the electrical conductivity of the milk. Adding water, proteins, sugar or insoluble salts reduces the concentration of ions and consequently reduces the conductivity of milk. This indicator is used to identify the milk of cows with mastitis. Electrical conductivity can also be used as an indicator of inflammation of the mammary gland (Fernando et al 1982; Paudyal et al 2020; Bonestroo et al 2022).

In our studies, the electrical conductivity was the same in the milk of both groups of cows (5.6 mS/cm). In our study, the conditions of chronic heat stress did not affect this indicator in dairy cows.

Active acidity

Active acidity of milk is a value that shows the concentration of free hydrogen ions in milk, that is, the degree of its acidity and alkalinity. In fresh milk, the active acidity is within the range of 6.6-6.8, that is, the milk has a slightly acidic reaction. As milk sours, this indicator gradually decreases. If the value is higher than 6.8, it indicates milk falsification (when substances are added to prevent souring) or mastitis (Santoso 2020; Tomovska et al. 2016). In our studies, this rate was 6.63 in both groups.

Marketability of milk is the quantity of milk sold to a dairy plant as a percentage of the milk produced in a year (Ilchuk et al. 2016). According to our data, in spring (in the reference group), the marketability of milk was 96.3%, and in summer (in the experimental group) it decreased to 96.1%.

Thus, under conditions of moderate heat stress there was a significant decrease in the content of MSNF, mass fraction of fat and protein ($P<0.05$) in milk. The mass fraction of lactose and minerals tended to increase.

A number of researchers note that even with moderate heat stress (with THI $\geq$72-78), there are significant changes in the body of milking cows, which can significantly affect the physiological state (Wolfenson et al. 2019), fertility and dairy productivity of cows (Skliarov et al. 2022; López-Gatius et al. 2020). In particular, along with an increased respiratory rate, increased heart rate and increased rectal temperature (Koshchavka et al. 2020; Mylostyvyi et al. 2021a), there are also significant changes in blood biochemical parameters (Bernabucci et al. 2010); the number of red blood cells and the hematocrit levels are low, the content of hemoglobin and the number of white blood cells are reduced (Baumgard et al. 2015; Revskij et al. 2019). It is likely that possible changes in the physiological state of

cows did not significantly affect the milk quality indicators studied and covered in this article. However, our previous studies (Mylostyvyi et al. 2021b) showed an increase in the concentration of free fatty acids, including saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) in the serum of cows under prolonged heat stress. In the case of negative energy balance (NEB) during extensive lipolysis of adipose tissue and the entry of non-esterified fatty acids into the bloodstream, the fatty acid profile of milk changes in dairy cows (Hammami et al., 2015). For example, this is due to an increased concentration of oleic (C18:1) and linoleic acid (C18:2) (Lu et al. 2013). The same trend in the composition of milk fat was observed by other researchers (Tian et al. 2016), who reported an increase in the concentration of EFA and PUFA in the milk of cows due to heat stress. Further in-depth study of the fatty acid composition of milk using the method of gas chromatography will provide valuable data necessary to find possible herd management strategies to maintain high milk quality under seasonal heat stress.

It should be noted that according to the regulatory document of Ukraine (DSTU 3662:2018 Cow's Raw Milk. Specifications), the tested milk of both groups met the standards specified in this document. In our opinion, this result can be associated with the activation of compensatory mechanisms in the body of milking cows, due to which it is possible to minimize the deviation of milk indicators from the physiological standards. These processes can be embedded genetically, which ensures the continued existence of the species and the survival of offspring. These mechanisms may be the subject of further scientific research.

CONCLUSION

During the period of continuous moderate heat stress in milking cows, a significant decrease in the content of milk solids-not-fat, mass fraction of fat and protein was observed. The mass fraction of lactose and minerals tended to increase. In general, milk quality indicators were within the physiological standards. It follows that the body of animals used internal compensatory mechanisms to normalize the composition of milk.

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Association of DGAT1 Gene Related to Flavor, Odor, Cholesterol, and Mineral in Indonesian Sheep

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ABSTRAK

Amri F, Harahap RS, Sumantri C, Inounu I, Depison, Alwi Y, Gunawan A. 2023. Asosiasi gen DGAT1 terkait flavor, odor, kolesterol, dan mineral pada domba Indonesia. JITV 28(2):122-128. DOI: http://dx.doi.org/10.14334/jitv.v28.i2.3152

Diacylglycerol acyltransferase 1 (DGAT1) merupakan salah satu kandidat gen potensial untuk perbaikan kualitas dan gizi daging domba Indonesia. Tujuan penelitian ini untuk mengidentifikasi keragaman gen DGAT1 pada SNP g. 8539 C>T serta kaitannya kandungan flavor dan odor, kolesterol, dan mineral daging domba Indonesia. Total sampel yang diidentifikasi sebanyak 254 ekor domba jantan berumur 10–12 bulan yang terdiri dari 20 ekor domba ekor gemuk (DEG), 107 domba ekor tipis (DET), 10 domba komposit garut (DKG), 10 domba *compass agrinak* (DCA), 10 domba *barbados cross* (DBC), 20 domba garut (DG), 27 domba jonggol (DJ), dan 50 domba lokal jambi (DLJ). Sebanyak 100 ekor domba diantaranya digunakan untuk analisis asosiasi gen DGAT1 dengan kandungan flavor dan odor, kolesterol, dan mineral. Identifikasi keragaman DGAT1|ALuI dianalisis dengan metode PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). Hubungan gen DGAT1 dengan parameter nilai gizi daging dianalisis dengan metode GLM (General Linear Model). Hasil penelitian menunjukkan keragaman gen DGAT1 bersifat polimorfik pada DET, DG dan DEG, sedangkan pada DCA, DBC, DLJ dan DKG bersifat monomorfik. Dua genotipe, CC dan CT ditemukan dalam DET, DG dan DEG. Gen DGAT1 berasosiasi secara signifikan (P<0.05) dengan kandungan flavor dan odor, yaitu senyawa 4-Ethyloctanoic acid (EOA). Genotipe CT memiliki nilai EOA yang lebih tinggi dibandingkan CC. Keragaman gen DGAT1 tidak ditemukan kaitannya dengan kandungan kolesterol dan mineral. Gen DGAT1 dapat dijadikan kandidat marka genetik untuk perbaikan kualitas flavor dan odor domba Indonesia.

Kata Kunci: Kolesterol, DGAT1, Flavor, Odor, Mineral

ABSTRACT

Amri F, Harahap RS, Sumantri C, Inounu I, Depison, Alwi Y, Gunawan A. 2023. Association of DGAT1 Gene related to flavor, odor, cholesterol, and mineral in Indonesian sheep. JITV 28(2):122-128. DOI: http://dx.doi.org/10.14334/jitv.v28.i2.3152

Diacylglycerol acyltransferase 1 (DGAT1) is a potential candidate gene for improving Indonesian lamb's quality and nutrient value. The study aimed to identify the diversity of the DGAT1 gene with SNP g.8539 C>T and its relation to Indonesian lamb's flavor and odor, cholesterol, and mineral. Total of 254 ten to twelve months old sheep consisted of 20 Javanese fat-tail sheep (JFTS), 107 Javanese thin-tail sheep (JTTS), 10 Garut composite sheep (GCS), 10 Compass agrinak sheep (CAS), 10 Barbados cross sheep (BCS), 20 Garut sheep (GS), 27 Jonggol sheep (JS), and 50 Jambi local sheep (JLS). One hundred sheep were used to analyze the association of the DGAT1 gene with flavor and odor content, cholesterol, and mineral. The diversity of DGAT1|*Alu*I was analyzed with the PCR-RFLP method. The association of the DGAT1 gene with the nutritional value of meat was analyzed using the GLM (General Linear Model) method. The results showed that the DGAT gene was polymorphic in JTTS, GS, and JFTS and monomorphic in CAS, BCS, JLS, and GCS. CC and CT genotypes were found in JTTS, GS, and JFTS. SNP g.8539 C>T of DGAT1 gene had a significant association (P<0.05) with flavor and odor, 4-Ethyloctanoic Acid (EOA). However, the DGAT1 gene had no significant association (P>0.05) with cholesterol and mineral. The DGAT1 gene might be marker-assisted selection for improving lamb flavor and odor in Indonesian sheep.

Key Words: Cholesterol, DGAT1, Flavor, Odor, Mineral

INTRODUCTION

Increasing meat consumption per capita is one of the focuses of Indonesia to improve the quality of Indonesian human resources. One potential source to meet this goal is lamb. Sheep are already famous in Indonesia, adapted well to the tropical climate, and are used to being kept by Indonesian people, especially in rural areas. Lamb meat consumers consider flavor and odor the most critical parameters of lamb quality. The leading causes of

sheepmeat odor are the two compounds: branched-chain fatty acid (BCFA), present in all fatty tissue, has been implicated as the cause of such flavors as, has indole, which originated from pastoral diets. Branched-chain fatty acids (BCFAs) consisted of 4-methyl octanoic (MOA), 4- ethyl octanoic (EOA), 4-methyl nonanoic (MNA) acids, 3-methylindole (MI), and 4-methyl phenol (MP) are the chemical compounds that are accepted as the main contributors to flavor and odor (Watkins et al. 2012).

However, it is vital to improve the quality of lamb to increase the acceptance and value of Indonesian lamb. Improvement in the quality of flavor and odor could also stimulate the development of the lamb market (Listyariniet al., 2018). Identifying genes related to lamb quality is the best way to accelerate the improvement of Indonesian lamb through a molecular selection approach. One gene with immense potency as a genetic marker for quality traits of sheep meat is the Diacylglycerol acyltransferase 1 (DGAT1) gene (Gunawan et al. 2019). The DGAT1 gene is located on the reticulum endoplasm membrane and is essential in the intricacy of Glycerol (TG) synthesis. DGAT1, located in adipose tissue, has a role in reserve metabolic energy (Fang et al. 2012; Patel et al. 2012; Chitraju et al. 2019). High activity of DGAT1 has been identified in the liver, adipose tissue, small intestine, and mammary gland (Fang et al. 2012).

Polymorphism of DGAT1 with mutation site in exon 16-17 (DGAT1|AluI) and its association with carcass characteristics in Iranian sheep had been reported by (Mohammadi et al. 2012). Moreover, the DGAT1 gene is also associated with fat composition in goat milk (Li et al. 2013; Zonabend König et al. 2017), saturated fatty acid levels (Hatta et al. 2019), and minerals in cows. In Indonesian sheep, DGAT1 is associated with carcass weight, meat quality and retail cut, fatty acids, and carcass characteristics (Gunawan et al. 2019).

Our previous study has not studied the relationship between DGAT1 gene polymorphisms with flavor and odor, cholesterol, and mineral traits in various breeds of Indonesian sheep. Though the variety of breeds also affects the flavor, odor, cholesterol, and mineral traits in the lamb quality. Therefore, the study has been established to estimate the diversity of the DGAT1 gene and the association of the DGAT1 gene to Indonesian lamb quality traits including, flavor and odor, cholesterol, and mineral.

MATERIALS AND METHODS

Samples

All experiments were approved by the Animal Ethics Commission of the IPB University (approval no.117-2018 IPB). The total of 254 rams used in this study were Javanesse fat-tail sheep (JFTS) (n=20), Javanesse thin-tail sheep (JTTS) (n=107), Garut composite sheep (GCS) (n=10), Compass Agrinak sheep (CAS) (n=10), Barbados cross sheep (BCS) (n=10), Garut sheep (GS) (n=20), Jonggol sheep (JS) (n=27) and Jambi local sheep (JLS) (n=50). Sheep aged 10–12 months with male sex are kept in a caged group. The feed provided during maintenance consisted of elephant grass and concentrate. One hundred sheep were used to analyze the association of the DGAT1 gene with flavor and odor content, cholesterol, and mineral. Data for analysis association study with flavor and odor were obtained from JFTS (n=10), JTTS (n=75), and JS (n=15), while for cholesterol consisted of JFTS (n=10), JTTS (n=45), GCS (n=10), CAS (n=10), and BCS (n=10), JS (n=15). In addition, data analysis for mineral content consisted of JS (n=15) and JTTS (n=85).

DNA extraction and PCR amplification

The longissimus dorsi samples were used for DNA extraction using the Geneaid gSYNC DNA Extraction Kit. The SNP g. 8539 C>T of the DGAT1 gene used in this study refers to Gunawan et al. 2019. A pair of primers used to amplify the DGAT1 gene were (F: 5'-CCT CTG CCT TCT TCC ATG AG-'3 and R: 5'-CAG TAC AGC AGC AAG TGG TG-'3) with PCR product of 466 bp (base pair). The PCR amplifications were performed in a 15 µl consisting of 1 µl DNA samples, 0.4 µL of primers (forward and reverse), 7.5 µL of MyTaq Red Mix, and 6.1 µL of deionized water. PCR amplification using the AB Systems with the initial denaturation at 95 °C for 1 min, then followed by 35 amplification cycles of primer annealing at 60 °C for 15 s, extension at 72 °C for 15 s, and final extension at 72 °C for 1 min. The PCR amplification product was electrophoresis using 1.5% agarose gel.

Genotyping using PCR-RFLP

Genotyping is done by PCR-RFLP technique using AluI cutting enzymes. Five L of amplicons were added to a mix of 0.9 L DW, 0.7 L tango buffer, and 0.4 L AluI enzyme restriction and incubated at 37 °C for 16 hours. The PCR-RFLP product was electrophoresed using 2.5% agarose gel. The DNA fragments that emerged were compared with a 100 bp marker. Genotyping is carried out based on the length of the DNA fragment. The genotype of DGAT1 consisted of CC: 466 bp; TT: 390, 76 bp; and CT: 466, 390, 76 bp.

Flavor and odor analysis

The flavor and odor parameters measured were 4-Methyloctanoic Acid, 4-Ethyloctanoic Acid, 4-Methylnonanoic Acid, Indole, 3-methyl, Phenol, 3-

methyl, Undecanoic Acid, and Phenol, 4-methyl. 500 g of loin samples were carried out for flavor and odor analysis. Flavor and volatile odor compounds were extracted using the Likens-Niceson method, which combines distillation and extraction with a solvent using Gas Chromatography-Mass Spectrophotometry (GC-MS).

Cholesterol content analysis

Cholesterol examination using the method: CHOD-PAP (Cholesterol] Oxidase Peroxidase Aminoantipyrine Phenol) with the principle: measurement of total cholesterol after oxidation and hydrolysis of colorimetric indicator enzymes, namely chinonimine produced and 4-amino antipyrine and phenol with hydrogen peroxide with the help of a peroxide catalyst. Measurements were made at a wavelength of 546 nm. The serum was mixed with cholesterol test reagent, incubated for 10 minutes at 37°C, then read the results. Absorbance readings with blank reagents were carried out for 60 minutes.

Mineral content analysis

The mineral content was analyzed according to AOAC (2015) Official Method 969.08. The longissimus dorsi was used for mineral content analysis. The parameters were analyzed, including iron (Fe), zinc (Zn), kalium (K), and selenium (Se).

Data analysis

Genotype frequency

Genotype frequency is the ratio of genotypes to the total population. Genotype frequency is calculated by comparing the number of genotypes with the population. The genotype frequency was calculated using the following formula:

$$Xi = \frac{\sum_{i=1}^{n} ni}{N}$$

Xii is frequency genotype ii, ni is the number of individual genotypes ii, and N is the number of samples.

Allele frequency

Allele frequency is the ratio of the number of alleles to all alleles in a population. The Allele frequencies were calculated using the following formula:

$$Xi = \frac{(2nii + \sum_{j \neq 1} nij)}{(2N)}$$

Where Xi is allele X-*ith* frequency, nii is the number of individual *ii* genotypes, nij is the number of individuals *ijth* with initial genotype, and N is the number of samples.

Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium is the balance value between genotype frequency and allele frequency. The suitability test between the expected genotype values and the calculated observations was calculated by chi-squared. Hardy-Weinberg equilibrium was calculated using the following formula:

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

Where X^2 is a chi-square value, O is the number of genotypes observed, and E is the number of genotypes expected.

Association analysis

The association between the DGAT1 gene and flavor, odor, cholesterol, and mineral content was analyzed using the General Linear Model (GLM) method with Minitab® 18 Software. The difference was considered statically significant if the p-value <0.05. Pairwise differences between genotype effects were tested by performing the Tukey Model. The formula model was used:

$$Yij = \mu + genotype_i + breed_j + e_{ijk}$$

Where Yij is the performance of the individual lamb for flavor, odor, cholesterol, and mineral content; μ is the average of flavor, odor, cholesterol, and mineral content for each trait; genotype*i* is the fixed effect of *i-th* genotype; breed*j* is the fixed effect of the *j-th* breed; *eijk* is the random error.

RESULTS AND DISCUSSION

DGAT1 gene diversity

The PCR amplification of the DGAT1 gene with mutation C>T in exon 17 was successfully carried out using a primer designed, and the results showed one genotyped CC (466 bp) (Figure 1).

The identification results of DGAT1 using PCR-RFLP with the restriction enzyme AluI showed 2 genotypes: a combination of C and T alleles, namely CC and CT. One band at 466 bp for CC homozygotes, two bands consisting of 390 and 76 indicates TT homozygotes, and three bands at 466 bp, 390 bp, and 76 bp for CT heterozygotes, as shown in Figure 2.

The diversity of the DGAT1 gene was analyzed using the formula for allele frequency, genotype frequency, and Hardy-Weinberg balance. The allele and genotype frequency values and the Hardy-Weinberg balance of the DGAT1 gene are presented in Table 1. The results showed that the C allele was the dominant allele in all sheep populations, whose frequency value ranged from 93-100%, while the T allele frequency was 0-7%.

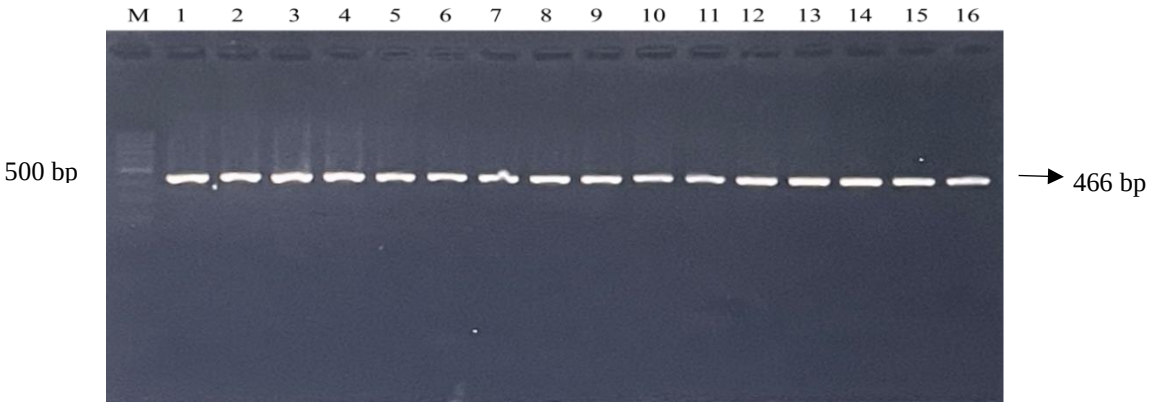

Figure 1. The results of amplifying the DGAT1 gene on 1.5% agarose gel. M= Marker 100 bp; 1-16= sheep samples

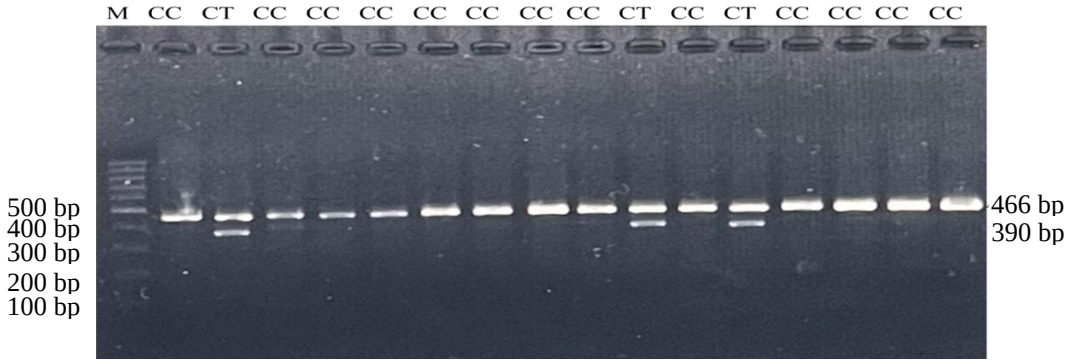

Figure 2. PCR-RFLP results of DGAT1| gene using AluI enzyme on agarose gel 2.5%. M= Marker 100 bp

This indicates that alleles are heterozygous for only 2% of the population. The low frequency of several alleles is most likely the result of random genetic drift (Star & Spencer 2013). The genotype frequencies obtained have different values; the CC genotype was 0.96, while the CT genotype was 0.04. Gene diversity is only found in Javanese fat-tail sheep (JFTS), Javanese thin-tail sheep (JTTS), and Garut sheep (GS). However, the DGAT1 in Compass agrinak sheep (CAS), Barbados cross sheep (BCS), Garut composite sheep (GCS), and Jambi local sheep (JLS) were monomorphic. This can be seen from the allele and genotype frequency values equal to 1.00. In this study, the population in the Hardy-Weinberg equilibrium value was 0.07. One of these factors can occur as a result of the genotype in the population being maintained/constant from generation to generation (Abramovs et al. 2020).

DGAT1 gene association with flavor and odor compounds

Association analysis of the DGAT1 gene with flavor and odor showed a significant association ($P < 0.05$) with 4-Ethyloctanoic Acid, while 4-methyl octanoic, 4-methyl nonanoic, 3-methylindole, 4-methyl phenol, 3-methyl phenol had no significant association with DGAT1. Individuals with the CT genotype had higher EOA values than the CC genotype (Table 2).

This study showed that DGAT1 had the most significant effect on phase I EOA metabolism in the liver. In contrast, the research report by (Listyarini et al. 2018) showed no relationship between EOA and CYP2A6 and KIFI2 genes. (Liu et al. 2012) stated that EOA is found in lamb, which plays a role in flavor compounds that are very important for flavoring in food.

The EOA compound has a relatively high molecular weight (C10H20O2) which has a positive impact because it is not volatile, so it can be used as an agent to repair unpleasant odors from other compounds. Another study related to EOA had a positive impact when the cooking test was carried out. The results did not affect the smell and taste of lamb (Resconi et al. 2013). Cattle reported that the DGAT1 lysine (L)>Alanine (A) polymorphism with an amino Acid substance at 232 K232A was associated with the amount of intramuscular fat that affected flavor and odor.

DGAT1 gene association with cholesterol

The association analysis of the DGAT1 gene with cholesterol showed no significant association ($P > 0.05$). Table 3 shows the results of the analysis. This is in line with the research that has no significant effect on cholesterols FAO products, some genes bound to acetyl-CoA will be incompletely oxidized to ketone bodies and cholesterol (Xue et al. 2018). The DGAT1 gene encodes

Table 1. Genotype and allele frequencies, and chi-square (χ^2) value of DGAT1 gene in Indonesian sheep

Sheep	N	Genotype frequency			Allele frequency		Chi-square (χ^2)
		CC	CT	TT	C	T	
JFTS	20	0.9 (18)	0.1 (2)	0.0	0.95	0.05	0.05^{ns}
JTTS	107	0.97 (104)	0.03 (3)	0.0	0.99	0.01	0.02^{ns}
JS	27	1.0 (27)	0.0 (0)	0.0	1.00	0.00	-
GCS	10	1.0 (10)	0.0 (0)	0.0	1.00	0.00	-
CAS	10	1.0 (10)	0.0 (0)	0.0	1.00	0.00	-
BCS	10	1.0 (10)	0.0 (0)	0.0	1.00	0.00	-
GS	20	0.85 (17)	0.15(3)	0.0	0.93	0.07	0.11^{ns}
JLS	50	1.0 (50)	0.0 (0)	0.0	1.00	0.00	-
Total	254	0.96 (246)	0.04 (8)	0.0	0.98	0.02	0.07^{ns}

N= Total samples; (..)= sample number which CC, CT, TT genotype; χ^2 table= 3.84. ^{ns}= not significant

Table 2. Genotype and association analysis of candidate genes with flavor and odor compounds

Parameters (ug/ul)	Genotype of DGAT1 ($\bar{x}$ ± SE Mean)			P value
	CC (97)	CT (3)	TT (0)	
4-*methyl octanoic* (MOA)	0.28±0.09	0.14±0.11	0.00±0.00	0.648 ^{ns}
4-*ethyl octanoic Acid* (EOA)	0.16±0.02^{b}	0.89±0.63^{a}	0.00±0.00	0.023*
4-*methylnonanoic* (MNA)	1.04±0.27	0.12±0.06	0.00±0.00	0.457 ^{ns}
3-*methylindole* (MI /Skatole)	0.03±0.01	0.02±0.01	0.00±0.00	0.838 ^{ns}
3-*methyl phenol* (MP)	3.22±0.69	1.11±1.11	0.00±0.00	0.767 ^{ns}
4-*methyl phenol (*MP)	0.001±0.0004	0.001±0.001	0.00±0.00	0.840 ^{ns}

$\bar{x}$= means of carcass traits; SE= standard error; (..)= the number of samples per genotypes; superscript a, b showed a significant difference at 5%; *=Significant at P<0.05; ^{ns}= Not significant. Different superscripts on the same row are different (P<0.05)

Table 3. Genotype and association analysis of candidate genes with cholesterol

Parameters (%)	Genotype of DGAT1 ($\bar{x}$ ± SE Mean)			P value
	CC (97)	CT(3)	TT(0)	
Cholesterol	7.17±2.58	6.59±2.41	0.00±0.00	0.686^{ns}

$\bar{x}$= means of carcass traits, SE= standard error, (..)= number of sample per genotypes, ^{ns}= not significant

Table 4. Genotype and association analysis of candidate genes with mineral

Parameters (mg/100 g)	Genotype of DGAT1 ($\bar{x}$ ± SE Mean)			P value
	CC (97)	CT (3)	TT (0)	
Fe	1.87±0.79	1.68±0.54	0.00±0.00	0.679^{ns}
Zn	2.62±0.99	2.47±0.51	0.00±0.00	0.807^{ns}
K	274.53±85.99	258.3±83.1	0.00±0.00	0.748^{ns}
Se	0.61±0.31	0.63±0.14	0.00±0.00	0.901^{ns}

$\bar{x}$= means of carcass traits, SE= standard error, (..)= number of sample per genotypes, ^{ns}= not significant

an enzyme containing 489 amino acids that catalyze the synthesis of TG by covalently linking TG to the substrate acetyl CoA. The study showed that acetyl-CoA incompletely binds to the DGAT1 gene during the formation of TG, which then leads to cholesterol oxidation. The relationship of cholesterol (VLDL and LDL) with triglycerides seen from forming very low-density lipoprotein (VLDL) will depend on the fatty acids available from adipose TG. VLDL is the primary lipoprotein in TG transport. TG synthesized by the liver is packaged into VLDL for distribution to peripheral tissues such as adipose tissue, heart, and skeletal muscle. The remaining VLDL is converted to intermediate-density lipoprotein (IDL) and then to (low-density lipoprotein) LDL by hepatic plasma triglyceride lipase (HTGL). Other genes associated with cholesterol control were reported by (Liang et al. 2020) in Chinese thin-tailed and fat-tailed sheep that the SREBF1 and SREBF2 genes affect cholesterol metabolism in the liver.

DGAT1 gene association with mineral

The association analysis of the DGAT1 gene polymorphism showed that the DGAT1 gene had no significant effect (P>0.05) on the mineral components, consisting of (Fe, Zn, K, and Se). Table 4 shows the results of the analysis.

However, the mean Fe obtained in our study ranged from 1.68-1.87 mg/ 100 g, Zn ranged from 2.47-2.62 mg/ 100g, K ranged from 258.3-274.53 mg/ 100g, and Se ranged from 0.61-0.63 mg/100 g. The data shows that the potassium content has the highest value of other mineral components. This happens because K is included in the macro-minerals. Humans' average macro-mineral requirement is more significant than 100 mg/day (Prashanth et al. 2015). Meanwhile, Fe, Zn, and Se are micro-minerals with the need for Fe and Zn (15 mg) and Se (55-70 mg) (Sigdel & Janaswamy 2020).

Minerals as essential macro and micronutrients function to maintain body resistance. Fe functions in oxygen transport in hemoglobin and is a component of several enzymes, including cytochromes, required for energy generation (Piskin et al. 2022). Deficiency of Fe and Se causes short bowel disease, which causes epithelial and mesenchymal dysfunction that affects the function and immune system of the gastrointestinal tract (Riaz & Mehmood 2012). Meat is a significant source of total iron (Fe) and heme iron, primarily of myoglobin and hemoglobin (Cabrera & Saadoun 2014). Potassium plays a role in maintaining cellular osmolarity and acid-base balance, as well as transmission of nerve stimulation and regulating heart and muscle function (Yamada & Inaba 2021). Zinc is an essential enzyme component, such as carbonic-anhydrase in red blood cells and dehydrogenase in the liver, and is a cofactor that increases enzyme activity. Zn deficiency causes dermatitis, slow growth, sexual maturity, infertility, and immunodeficiency (Khanam 2018).

CONCLUSION

The DGAT1 gene was polymorphic in JFTS, JTTS, and GS, while in CAS, BCS, JLS, JS, and GCS were monomorphic. The CC genotype is still dominant in Indonesian sheep. The DGAT1 gene was significantly associated with flavor odor, namely 4-Ethyloctanoic Acid (EOA). Sheep inheriting the CT genotype had higher EOA compared to the CC genotype. However, there is no significant association between DGAT1 diversity with cholesterol and mineral content. The diversity of the DGAT1 gene might contribute to the flavor odor compound in Indonesian sheep.

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Production Performance and Sperm Characteristics of Quail (*Coturnix-coturnix japonica*) with Different Concentrations of Yolk Immunoglobulin

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ABSTRAK

Agasi SY, Ulupi N, Afnan R, Arifiantini RI. 2023. Performa Produksi dan Karakteristik Sperma Puyuh Jantan (*Coturnix-coturnix japonica*) dengan Konsentrasi Immunoglobulin Yolk Berbeda. JITV 28(2):129-135. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3173.

Puyuh diklasifikasikan menurut kekebalannya terhadap konsentrasi IgY yang berbeda. Konsentrasi IgY puyuh berkisar antara 0 sampai 1200 µg ml^{-1} dan konsentrasi IgY ini dapat memengaruhi produksi dan kinerja reproduksinya. Penelitian ini bertujuan untuk menganalisis konsentrasi IgY pada puyuh jantan dan membandingkan performa produksi dan reproduksi pada konsentrasi IgY yang berbeda. Empat puluh dua ekor puyuh jantan usia lima minggu digunakan dalam penelitian ini, 29 puyuh dengan konsentrasi IgY rendah (210-393 µg ml^{-1}) dan 13 puyuh dengan konsentrasi IgY sedang (403-564 µg ml^{-1}). Performa produksi yang diamati adalah konsumsi pakan, bobot badan awal, bobot akhir, pertambahan bobot badan, konversi pakan, morbiditas, dan mortalitas. Bobot testis dan kualitas semen secara makroskopis dan mikroskopis diuji untuk sifat-sifat reproduksi. Perbedaan performa produksi dan reproduksi masing-masing kelompok dianalisis menggunakan *independent-sample t-test*. Hasil penelitian menunjukkan puyuh jantan dengan konsentrasi IgY berbeda memiliki performa produksi yang sama (P>0,05). Hal tersebut dapat diartikan puyuh jantan dengan konsentrasi IgY rendah dan IgY sedang sama baiknya. Berat testis, warna dan pH semen puyuh sama, pada kedua kelompok konsentrasi IgY yang berbeda. Konsistensi semen IgY rendah lebih kental dibandingkan dengan konsentrasi IgY rendah. Puyuh dengan konsentrasi IgY rendah memiliki konsistensi semen yang lebih baik, hal ini berhubungan dengan ukuran testis dari puyuh dengan konsentrasi IgY rendah, lebih berat dibandingkan puyuh dengan IgY sedang. Konsistensi ini berhubungan dengan konsentrasi sperma di dalam semen tersebut. Hasil penelitian menyimpulkan konsentrasi IgY pada puyuh jantan tidak memengaruhi performa produksi. Puyuh dengan konsentrasi IgY rendah memiliki konsistensi sperma yang lebih kental dan konsentrasi sperma yang lebih tinggi.

Kata Kunci: Konsentrasi IgY, Performa, Puyuh, Karakteristik Semen

ABSTRACT

Agasi SY, Ulupi N, Afnan R, Arifiantini RI. 2023. Production Performance and Sperm Characteristics of Quail (*Coturnix-coturnix japonica*) with Different Concentrations of Yolk Immunoglobulin. JITV 28(2): 129-135. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3173.

Quails are classified according to their immunity to different IgY concentrations. Quail IgY concentrations range from 0 to 1200 µg ml-1, and these IgY concentrations may affect production and reproductive performance. This study aimed to analyze IgY concentrations in male quail and to compare production and reproductive performance at different IgY concentrations. Forty-two male quail at five weeks of age participated in the study, including 29 quail with low IgY concentration (210-393 µg ml^{-1}) and, 13 with intermediate IgY concentration (403-564 µg ml^{-1}). The observed productive performances were feed intake, initial body weight, final body weight, weight gain, feed conversion, morbidity, and mortality. In addition, this study observed testicular weight and macroscopic and microscopic semen quality for reproductive traits. Differences in production and reproductive performance of each group were analyzed using an independent-sample t-test. The result showed that male quail with different IgY concentrations were equal in all productive traits (P>0.05). This means that male quail with low IgY and medium IgY concentrations are equally good. Testicular weight, semen color and pH were identical at different IgY concentrations. Quails with low IgY concentrations have better sperm consistency, which is related to the weight of the testes, which are heavier in quails with low IgY concentrations than in those with moderate IgY concentrations. This consistency is related to the concentration of sperm in the semen. The result concludes IgY concentrations in male quail did not affect production performance. Quails with low IgY concentration had thicker sperm consistency and higher sperm concentration.

Key Words: IgY Concentration, Performance, Quail, Semen Characteristics

INTRODUCTION

Quail has rapid growth, earlier sexual maturity, high egg production rate, short generation interval (3-4 generations per year), low space requirements, lower feed requirements, short incubation period of hatching eggs, lower feed costs, and lower susceptibility to common chicken diseases (Rahman et al. 2016). Quails were once small birds for toys, but are now used for commercial egg and meat production (Khairani et al. 2016). High quail productivity depends on several factors, including seed, health, feed, environment, and management. Quail productivity can be evaluated by body weight gain, feed efficiency, egg production, disease resistance, and stress. Immunity is influenced by the immune system, which is supported by the function of immune cells to maintain immunity against pathogens that can infect the body. The immune system plays an important role in fighting various diseases (van Seventer & Hochberg 2017).

According to van Seventer & Hochberg (2017), body resistance is the body's response to bacteria, viruses, fungi and parasites, which is influenced by many factors such as feed, husbandry management and genetics. According to Spillner et al. (2012), some poultry species are very sensitive to contact with foreign antigens, which affects the immune system and the production of immunoglobulins (IgY). Immunoglobulin (Ig) is the first substance identified as a molecule in serum that can neutralize various foreign bodies or microorganisms that cause infections. Poultry immunoglobulin consists of IgA, IgM, and IgY. Yolk immunoglobulin plays a role in the poultry's main system, which can inhibit pathogenic bacteria (Zhang et al. 2019). The higher the IgY concentration, the better the immune system (Setyawati 2018). One indicator to measure the immune system of quail is to examine the IgY content in the animals' serum. Immunoglobulins found in blood serum and egg yolk (Pereira et al. 2019). IgY concentration in blood serum ranges from 5-15 mg mL^{-1} (Gaetani et al. 2017).

High resistance of the body improves production performance (Regar et al. 2013). Reproductive performance depends on the quality of both female and male animals. Males for breeding purposes must have high reproductive performance, including good semen quality. Semen quality plays an important role in fertility (Modupe et al. 2012). Semen quality of poultry can be assessed by macroscopic and microscopic assessments of semen. Macroscopic assessments include semen volume, color, consistency, and pH, while microscopic assessments include mass movement, motility, viability, concentration, and sperm abnormalities (Malik et al. 2013; Elagib et al. 2012). Several researchers have studied the relationship between IgY concentration and cock performance, including Ariyanti et al. (2019) and Setiawan et al. (2021). Ariyanti et al. (2019) reported that Sentul chickens with high IgY concentrations (> 9.30±0.45 mg mL^{-1}) potentially fertilize more hens. Setiawan et al. (2021) reported that IPB-D1 chickens with high IgY concentrations (>9.36±2.88 mg mL^{-1}) had better sperm motility and viability compared to low IgY concentrations. Both researchers found that IgY concentration did not affect performance, but high IgY concentrations in chickens showed the potential to fertilize more hens and better sperm motility and viability compared to chickens with low IgY. The aim of this study was to find out whether IgY concentrations in quail also lead to the same results. Therefore, the performance and semen quality as well as the fertilization potential of female quail were investigated in this study.

MATERIALS AND METHODS

Approval of the animal ethics committee

This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Biomedical science, Bogor Agricultural University (IPB), under approval number 046/KEH/SKE/XI/2022.

Time and place

The study was conducted from October to December 2021. Quail rearing was conducted in the field laboratory of the Department of Breeding and Genetics, Faculty of Animal Husbandry, Bogor Agricultural University. IgY concentration tests were performed in the integrated laboratory of the Division of Animal Diseases and Public Veterinary Medicine (IPHK), and semen quality tests were performed in the laboratory of the Reproductive Rehabilitation Unit (URR), Division of Reproduction and Obstetrics, School of Veterinary Medicine and Biosciences, IPB University.

Examination of IgY concentration of male quails

The 42 male quails used in this study were five weeks old. The initial body weight of the quails ranged from 100-137 g $head^{-1}$. The determination of total IgY concentration in blood serum was performed using the indirect enzyme-linked immunosorbent assay (ELISA) method according to Murai et al. (2016). Blood was collected from the brachial vein of the wing using a 0.5-mL syringe. The total IgY values of all samples were averaged. Quails with IgY concentration above the average are classified as quails with high IgY concentration. Quails with IgY concentration equal to or below the average are classified as quails with low IgY concentration.

Quail rearing

The quails were housed in wire mesh cages measuring 15 x 30 x 25 cm^3 and were provided with food and water in each cage. The cages were equipped with a 7-watt lamp for illumination at night. New Hope P-100 laying quail feed was used in this study. New Hope P-100 contains a maximum moisture content of 13%, minimum protein of 20%, minimum fat of 7%, maximum ash of 7%. Quails were fed at 7:00 am and 4:00 pm and received drinking water ad libitum. Quails and feed residues were weighed weekly to determine body weight gain and feed consumption.

Evaluation of quail performance.

Quail performance at different IgY concentrations was evaluated were feed consumption (g) was calculated from the number of feedings minus feed leftovers. Final body weight (g) is the body weight of the quail at the end of the study. Body weight gain (g) was determined by calculating the final and initial body weight. The feed conversion ratio was determined from the amount of feed consumed divided by the amount of weight gain. Morbidity (%) was determined by dividing the number of sick quail by the total number of quail and multiplying by 100%. Mortality (%) was determined by dividing the number of quail that died by the total number of quail and multiplying by 100%.

Testing of quail semen properties

Semen collection

Quail sperm collection was performed on 13-week-old quails. Sperm collection was performed using the epididymal collection method. Quails were slaughtered and dissected, and both testes were freed from other tissues and weighed with a digital scale. Sperm was collected from the right and left epididymis (Ouennes et al. 2019).

Evaluation of the semen

The semen was examined macroscopically and microscopically. The macroscopic evaluation included color, consistency and pH. Due to the technique of sperm collection from the epididymis, sperm volume was not determined in this study. The pH of the semen was measured using a special pH indicator paper (Merck scale 6.4-8). Five µl of semen was dropped onto the pH paper and allowed to stand for 15-30 seconds. The consistency of the semen was classified as thin, medium, and thick, and the color of the semen was observed visually.

Microscopic examination of semen, including motility, viability, concentration, and sperm abnormalities, was performed using a binocular microscope (Olympus CX23). Sperm motility was examined by mixing 2 µl of semen with 20 µl of physiological saline. The solution was homogenized and covered with a coverslip. The slides were viewed under a microscope at 400× magnification. Sperm motility was assessed by estimation from 5 fields of view by comparing the number of sperm moving forward with other sperm movements. Motility values were expressed as percentages. Sperm viability was determined by mixing 5 µl of semen with 50 µl eosin-nigrosin solution. The mixture was homogenized, then a test preparation was made and dried on a heating table for 10-15 seconds. The preparations were viewed under a microscope at 400× magnification. The percentage of live sperm was counted in 10 fields of view, and the minimum number of cells had to be >200. Live sperm do not absorb color, while dead sperm stain purple. The percentage of live sperm is calculated according to the formula: Number of live sperm divided by the total number of sperm multiplied by 100%.

When observing sperm abnormalities, the same staining is used to examined live sperm. The percentage of abnormal and normal sperm is determined in 10 fields of view with a minimum cell count of 200 cells (Hambu et al. 2016). The percentage of abnormal sperm is calculated using the formula number of abnormal sperm divided by the total number of sperm and then multiplied by 100%. Sperm concentration was calculated using a Neubauer chamber. The semen was diluted 500 times with formol saline (2 µL semen in 998 µL formol saline). The mixed solution was placed in the counting chamber and viewed under a microscope at 400× magnification. Sperm counts were performed on five counting boxes. The sperm concentration was calculated using the formula: Number of sperm counted x 25 x 10^6.

Data analysis

Data on production performance (feed consumption, initial body weight, final body weight gain, feed conversion), testicular weight, and semen quality (color, pH, sperm consistency, motility abnormalities, and sperm concentration) were analyzed by independent-sample t-test (Mishra et al. 2019) using the SAS program. Data on overall morbidity and mortality were analyzed descriptively.

RESULTS AND DISCUSSION

The results of indirect Elisa IgY measurement in quail ranged from 210 to 564 µg ml^{-1}. Quail IgY

according to Murai et al. (2016) ranged from 0 to 1200 µg ml^{-1}. The IgY results have only "low IgY" and "moderate IgY" categories. Quail with low IgY had a value of 210-393 µg ml^{-1} with a total of 29 quail and quail with moderate IgY had a value of 403-564 µg ml^{-1} with a total of 13 quail.

Male quail performance

The results of the analysis of male quail performance are presented in Table 1. The performance of male quail with different IgY concentrations showed no differences (P>0.05) in feed consumption, initial body weight, final body weight, weight gain, and feed conversion. This result means that male quails with low and medium IgY concentrations performed equally well. The feed conversion ratio of male quail with low IgY concentration and male quail with medium IgY concentration has a value of 5.98 ± 2.49 and 5.37 ± 2.08, respectively. This feed conversion ratio is in the high category. This is due to the fact that quail enter the production phase at nine weeks of age, so the digested feed nutrients are used for reproductive organ development rather than growth (Panjaitan et al. 2012).

The results of this study are better than those of Dewi et al. (2016), who put the feed conversion value of male quail at 6.31. Khalil (2015) indicated that the feed conversion value of quail is 3.3-4.9. The lower the feed conversion value, the more efficient the feed conversion and vice versa. Mortality of quail with low IgY concentration was 3.45% (1 of 29 birds) higher than quail with medium concentration. The mortality was not due to quail disease, but was due to cage wire entrapment. Morbidity of male quail with different IgY concentrations was 0%. During the study, no quail showed disease symptoms such as watery eyes, matted and dull plumage, and lethargy. Quail are known to be highly resistant to disease (Rabiu-Mohammed & Ejiofor 2015). The results of this study confirm reports by Ariyanti et al. (2019) and Setiawan et al. (2021) that low or high concentrations of IgY do not affect production performance in chicken and quail.

Testicular weight

The results of the analysis of testicular weights of quails are shown in Table 2. The testicles are the organs that produce sperm. Testicular weight provides information about sperm productivity. The results showed that testicular weights did not differ between quails with low and medium IgY concentration (P>0.05), nor between right and left testicular weights. Testicular size is used as a supportive indicator of sperm production. A positive correlation between testis, body weight, and sperm production has been found in some animals (Indriastuti al. 2020; Perumal 2014). Testicular weight is dependent on age, breed, season, and diet. The male quail used in this study were of the same age, breed, and diet. As quail age, body weight increases and so does the weight of the organs in the body, which include the testes. The testes consist internally of testicular tubules (85%-95% of the testicular volume), where spermatogenesis takes place. The larger the volume of testicular tubules in the testis, the more sperm are produced. The walls of the testicular tubules are

Table 1. Production performance of 9-week-old male quails with different IgY concentrations

Variables	Low IgY	Moderate IgY
Feed consumption (g $head^{-1}$ $week^{-1}$)	160.78±27.17	165.79±7.52
Initial body weight (g $head^{-1}$)	117.14±9.39	114.69±12.91
Final body weight (g $head^{-1}$)	144.82±12.75	145.54±15.15
Body weight gain (g $head^{-1}$ $week^{-1}$)	26.90±10.29	30.85±10.38
Feed conversion	5.98±2.49	5.37±2.08
Morbidity (%)	0	0
Mortality (%)	3.45	0

Table 2. Testicular weight of quail with different IgY concentrations.

Testis	Low IgY (n=6)	Moderate IgY (n=6)
Right (g)	2.16±0.63	1.94±0.37
Left (g)	2.52±0.37	1.92±0.73
Mean (g)	2.34	1.93

composed of connective tissue and germinal epithelial tissue, which plays a role in spermatogenesis. Testicular weight is closely related to sperm concentration. The results showed that male quail with low IgY concentrations tended to have higher testicular weight. This fact is expected to influence the amount of sperm produced.

Macroscopic semen quality

The results of the macroscopic analysis of quail sperm quality are shown in Table 3. The color and pH of quail semen with different IgY concentrations did not differ (P>0.05). Quail with low IgY concentration had thicker sperm consistency than quail with medium IgY concentration (P<0.05) (Table 3). Semen consistency and color in poultry, including chickens, can describe sperm concentration (Junaedi et al. 2016). Semen that is thick and has a milky white color has a high sperm concentration and vice versa. The higher the sperm concentration, the more intense the color (Sujoko et al. 2009).

The acidity (pH) of the semen determines the life status of the sperm within it. A high or low semen pH leads to a faster sperm death (Sujoko et al. 2009). Semen pH is the same for low IgY and medium IgY, namely 7.3. Fresh semen from poultry is usually slightly alkaline, with an average pH between 7.0 and 7.6. Sperm pH is influenced by several factors, including sperm activity in the breakdown of fructose, which lowers pH. The high activity of sperm in decomposing energy sources from fructose increases the production of lactic acid in sperm, making the pH more acidic. Table 3 shows a significant difference in sperm consistency. Quail with low IgY concentrations have better sperm consistency, which is related to the fact that the testes of quail with low IgY concentrations are heavier than those of quail with medium IgY concentrations. This consistency is related to the concentration of sperm in the semen.

Motility, viability, and abnormalities of quail sperm did not differ between quails with different IgY concentrations (P>0.05). This result is due to the fact that all quail were in good health at the time of semen collection. Relatively healthy quails produce the same production. The sperm motility of quail in this study ranged from 46.67 ± 15.06 to 51.67 ± 24.63, which is lower than the study of Lesmono et al. (2017) on six-week-old quail, which ranged from 80% to 88%. Sperm viability ranged from 58.71±12.31% to 66.35±9.54%. Sperm viability is a crucial indicator for assessing good or poor sperm quality. In this study, quail sperm abnormality was very high and ranged from 38.97±13.65 to 52.57±26.52%. According to Lesmono et al. (2017), sperm abnormality in quail is ±7.4%. Abnormal sperm correlate with motility, fertility and hatchability in poultry (Feyisa et al. 2018).

The sperm concentration of quail with low IgY concentration (1521.88±18.92x10^6) was higher than quail with medium IgY concentration (831.25±388.00x10^6). The sperm concentration in this study was higher than that reported by Chelmonska et al. (2008), which was 120-312 x 10^6 ml^{-1}. Chelmonska et al. (2008) reported that quail sperm concentration can reach 2240-2640 × 10^6 ml^{-1} with appropriate male selection. High sperm concentration increases the potential of females to be inseminated. The amount of quail sperm required for artificial insemination is not known with certainty. Thélie et al. (2019) indicated that using 15-60 x10^6 sperm per insemination results in a

Table 3. Macroscopic quality of quail semen with different IgY concentrations

Variables	Low IgY	Moderate IgY
Semen color	2±0.0	1.5±0.55
Semen pH	7.3±0.29	7.3±0.38
Semen consistency	2±0.0^b	1.17±0.41^a

Different letters in the same row indicate significant differences (P<0.05). Color 1= cloudy white, 2= milky white; Consistency 1= medium, 2= thick

Table 4. Microscopic quality of quail semen with different IgY concentrations

Variables	Low IgY	Moderate IgY
Sperm motility (%)	51.67±24.63	46.67±15.06
Sperm viability (%)	66.35±9.54	58.71±12.31
Sperm abnormalities (%)	38.97±13.65	52.57±26.52
Sperm concentration (x10^6 mL^{-1})	1521.88±18.92b	831.25±388.00a

Different letters following numbers in the same row indicate significant differences (P<0.05)

high fertility rate of up to 80%. The high sperm concentration in quail with low IgY concentration is influenced by testicular weight and sperm consistency.

Microscopic semen quality in quail with different IgY concentrations

The results of the microscopic quality analysis of quail semen are shown in Table 4. Quail sperm quality was varied in this study. These differences may be explained by individual variations as reported by Indriastuti et al. (2020) in Bali cattle. When collected by abdominal massage, semen quality was also varied. Breed, location, diet, age, and climate differ as sperm parameters may varied depending on these factors (das et al. 2016; Kuzlu & Taskin 2017; Mavi et al. 2019). This is also due to the uneven distribution of data in each group. Testicular weights from this study were positively correlated with better sperm concentration in quail. This fact was evident in quail with low IgY concentrations. Quails with low IgY concentrations have a weaker immune system. Therefore, to maintain the immune system of quail, better maintenance management, feeding management, and biosecurity for quail with low IgY concentrations are needed to maintain their immune system and use them for breeding.

CONCLUSION

There is no difference in the production performance of male quail with low and medium IgY concentrations. Quails with low and medium IgY had the same testicular weight, semen color and pH. Semen consistency and sperm concentration of quails with low IgY were better than quails with medium IgY.

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Effect of Zinc Supplementation in the Diet on Sikumbang Janti Female Duck Performance, Carcass, Digestive Organs, and Intestinal Morphology

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ABSTRAK

Rusli RK, Amizar R, Zurmiati, Ananda, Darmawan A, Subekti K, Khalil. Pengaruh suplementasi zinc dalam pakan terhadap performa, karkas, organ pencernaan, dan morfologi intestinal bebek Sikumbang Jati betina. JITV 28(2):136-142. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3209.

Penelitian bertujuan untuk mengevaluasi pengaruh suplementasi Zn pada ransum itik Sikumbang Janti betina terhadap performa, karkas, organ pencernaan, dan morfologi usus. Penelitian menggunakan 96 ekor itik betina umur 8 minggu dipakai sebagai materi percobaan. Penelitian ini menggunakan rancangan acak lengkap dengan empat perlakuan (0, 30, 60, dan 90 mg Zn/kg), masing-masing perlakuan diulang sebanyak empat kali. Parameter yang diukur performa, karkas, organ pencernaan, dan morfologi usus. Hasil penelitian menunjukkan bahwa suplementasi Zn berpengaruh nyata (P<0,05) meningkatkan bobot badan, pertambahan bobot badan, efisiensi penggunaan ransum, bobot karkas, menurunkan lemak perut, dan meningkatkan ukuran morfologi usus, namun tidak mempengaruhi (P>0,05) konsumsi pakan, persentase karkas, dan ukuran organ pencernaan. Sebagai kesimpulannya, suplementasi 60 mg Zn/kg dalam ransum dapat memperbaiki performa itik Sikumbang Janti betina yang dipelihara umur 8-16 minggu.

Kata Kunci: Suplementasi Zn, Performa Pertumbuhan, Morfologi Usus, Itik Lokal, Organ Dalam

ABSTRACT

Rusli RK, Amizar R, Zurmiati, Ananda, Darmawan A, Subekti K, Khalil. Effect of zinc supplementation in the diet on Sikumbang Janti female duck performance, carcass, digestive organs, and intestinal morphology. JITV 28(2):136-142. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3209.

The research aimed to evaluate the effect of Zn supplementation in diet of Sikumbang Janti female duck on its performance, carcass, digestive organs, and intestinal morphology. The study used 96 female ducks aged 8 weeks. This research used a completely randomized design with four treatments (0, 30, 60, and 90 mg Zn/kg), each treatment was repeated four times. Performance, carcass, digestive organs, and intestinal morphology were observed. The results showed that Zn supplementation significantly (P<0.05) affected body weight, body weight gain, feed conversion ratio, carcass weight, abdominal fat, and intestinal morphology, but it did not affect (P>0.05) feed consumption, carcass percentage, and digestive organs. In conclusion, supplementation of 60 mg Zn/kg in the diet improved performance, intestinal morphology, and the health of visceral of Sikumbang Janti female ducks aged 8 to 16 weeks.

Key words: Dietary Zn Supplementation, Growth Performance, Intestinal Morphology, Local Duck, Visceral

INTRODUCTION

Ducks are one of the potential egg and meat-producing types of poultry after chicken commodities. In the West Sumatra province, the population of ducks in 2021 was around 1,185,955 birds (Badan Pusat Statistik 2022), spread throughout rural and urban areas. One of the potential indigenous ducks in Payakumbuh City, West Sumatra, is Sikumbang Janti duck.

However, the development of the Sikumbang Janti duck population in West Sumatra faces various obstacles, including the dominance of smallholder duck farms with extensive systems and low-quality feed. The extensive duck-rearing system is heavily dependent on the availability of quality feed. A lack of quality feeds will cause performance to decrease, as well as an increase in disease and mortality rates. In addition, to feed quality, productivity and mortality are closely related to environmental conditions (e.g., high relative humidity and temperature). This condition must be overcome immediately in terms of developing the Sikumbang Janti duck population. Moreover,

information on the status of Zn mineral in common feed duck rise in West Sumatera is not available. Therefore a study to determine the Zinc level required by Sikumbang-janti duck is important. One of the efforts that can be conducted is supplementing zinc (Zn) minerals in the ration to fulfill the trace mineral requirement and increase feed quality

Zink (Zn) is a micro-mineral that plays various processes, i.e., gene regulation, cell proliferation, cofactor enzyme, immune responses, defense against oxidative stress (Maret 2013; Marreiro et al.. 2017; Hidayat et al.. 2020), growth (Liu et al.. 2018), intestine health, eqq quality (Fan et al.. 2022) and reproduction (Chand et al.. 2014). Previous research found that consuming Zn can enhance the productivity and immune systems of Longyan ducks, Magelang duck, and Pekin ducks (Attia et al.. 2013; Darmawan et al.. 2013; Zhang et al.. 2021). However, there has been no report on the supplementation of Zn in the diet of Sikumbang Janti female ducks. Therefore, this study aimed to evaluate the effect of Zn supplementation in diet of Sikumbang Janti female duck on its performance, carcass, digestive organs, and intestinal morphology.

MATERIALS AND METHODS

Animal, design, and diet

This research was approved for experimental animals by the Research Ethics Committee of the Faculty of Medicine Universitas Andalas (No. 31/UN.16.2/KEP-FK/2023). This research used 96 Sikumbang Janti female ducks aged 8 weeks (average weight of 752.41±81.99 g/bird). The Sikumbang Janti female ducks were acquired from a commercial farmer from Payakumbuh City, West Sumatra, Indonesia. The observation was conducted for 8 weeks and used a completely randomized design of 4 treatments with 4 replicates (6 ducks/replicate). Ducks were distributed at random into cages (1.5 x 1.5 m). A drinker tube and a bucket feeder were installed in the cage to supply feed and water. The Zn used was 75% ZnO (Zn-O-India). The ration was prepared based on Sinurat, (2000), with a 17% crude protein, and 2700 metabolizable energy. Table 1 lists the treatment diet's ingredients and composition. The treatments design are presented in Table 2.

Table 1. Feed ingredient and nutrient content of control diet

Ingredients	%
Corn	55.00
Rice bran	15.40
Soybean meal	18.00
Fish meal	8.50
$CaCO_3$	2.50
Top mix[1]	0.50
Dl-Methionine[2]	0.10
Total	100.00
Metabolizable Energy, (Kcal/kg)	2744.55
Crude Protein, (%)	17.36
Crude Fibre, (%)	3.63
Crude Fat, (%)	1.58
Available Phosphorus, (%)	0.59
Methionine, (%)	0.54
Lysine, (%)	1.22
Zn, (mg/kg)	29.59

[1]Provides per kilogram of diet = Vitamin A 12.000 IU; Vitamin D3 2000000 IU; Vitamin E 8000 IU; Vitamin K_3 2000 mg; Vitamin B_1 2000; Vitamin B_2 5000 mg; Vitamin B_6 500 mg; Vitamin $B1_2$ 1.200 µg; Vitamin C 25.000 mg; Ca-D-Pathotenate 6.000 mg; Niacin 40000; Cholin Chloride 10000 mg; Lysine 30.000 mg; Methionine 30000 mg; Manganese 120000 mg; Iron 20000 mg; Zinc 100 mg; Iodine 200 mg; Cobalt 200 mg; Copper 4.000 mg; Santoquin 10000 mg; Growth promoter 1300000, [2]Dl-Methionine (Shandong Nhu Amino Acid Co. LTD)

Table 2. Zinc supplementation experimental diets

Treatments	Description	Total Zn in the diet (mg/kg)
Z0	Control feed	29.59
Z1	Z0 + 30 mg Zn/kg	59.59
Z2	Z0 + 60 mg Zn/kg	89.59
Z3	Z0 + 90 mg Zn/kg	119.59

Variables observed

Feed consumption (FC), body weight gain (BWG), and feed conversion ratio (FCR) were recorded weekly at each replication. A digital weighing scale measured feed and body weight (BW). Carcass, and digestive organs followed the procedure described by Mutia et al. (2017), at the end of the research (16 weeks), Sikumbang Janti female ducks (one duck per repetition) were slaughtered to obtain carcass, digestive organs. The observed variables included carcass, abdominal fat, proventriculus, ventriculus, pancreas, liver, spleen, heart, bile, small intestine, ceca, and colon. A digital balance (Osuka-HWH®, Japan). Intestinal morphology. Intestinal sample preparation was carried out by following the methods of Chiou et al. (1999). the ileum intestinal sample was cut 2 cm long and then soaked in 10% formalin to make preparations. Villus wide (VW), villus height (VH) and crypt depth (CD) were determined using an Olympus CX 21 microscope with 4x magnification. After the histology of the intestine was found as expected, a photo was taken. Minimum measurements were made three times per slide made for each parameter. additionally, ImageJ Ink software made Villus wide, villus height, and crypt depth measurements on a computer. A computer determined the μm size standard in advance where the magnification value used was in units of length (μm). The μm unit number obtained was then used as a standard in calculating the villus wide, villus height, and crypt depth on a monitor screen.

Data analysis

The data obtained were analyzed by analysis of variance, and differences between treatments followed Duncan's multiple range test. The means data were provided with SEM and P values.

RESULTS AND DISCUSSION

Performance

The impact of Zn supplementation on the performance of Sikumbang Janti female ducks (Table 3) showed that Zn supplementation at 30 mg/kg significantly ($P<0.05$) decreased FCR while increasing BW and BWG compared to the control group. The effects of zinc supplementation at 30 mg/kg on FCR, BW, and BWG were equivalent to those of supplements at 60 and 90 mg/kg. During the trial, adding Zn did not impact FC ($P>0.05$).

The production performance (BW, BWG, and FCR) of the Sikumbang Janti female duck, was significantly increased by an increase in the Zn level. In this study, the average value of total Zn consumed for Z0, Z1, Z2, and Z3 was 177.46 mg/bird (29.59 mg/kg), 360.35 mg/bird (59.59 mg/kg), 536.34 mg/bird (89.59 mg/kg), and 713.62 mg/bird (119.59 mg/kg), respectively. These results were supported by previous studies showing that the mineral Zn can meet the needs of trace minerals that can support production performance, reproduction, immunity, and the normal development of feathers and bones (Abd El-Hack et al. 2017; Hidayat et al. 2020; Jafari et al. 2021; Hidayat et al. 2021; Zhu et al. 2022). This was also in line with other studies which reported that Zn supplementation on the Pekin duck diet increased BW and WG, decreased FCR, and did not affect FC (Attia et al. 2013; Wu et al. 2019; Xie et al. 2021). However, Attia et al. (2013) reported that adding 120 ppm Zn to the white Pekin duck diet decreased BWG.

Zn is an essential element of digestive enzymes and other enzymes such as hydrolases, oxidoreductases, transferases, lyases, ligases, and isomerases (Park et al. 2004). Therefore, Zn in feed aids in the digestion and metabolic processes of proteins, lipids, and carbohydrates into substrates that are easily absorbed in the intestine and stored in the tissue (Azad et al. 2020). According to Azad et al. (2020), the addition of 50 mg Zn to the broiler's diet is sufficient for proper growth up to 28 days of age. Similarly, adding zinc to poultry feed increases their ability to produce antibodies. Thymulin, the thymus hormone, regulates T lymphocytes by promoting T lymphocyte maturation and activating lymphocytes. Zn is a crucial component of this hormone (Weyh et al. 2022). Zn supplementation triggers the development of lymphocytes which can reduce stress in poultry as indicated by a decrease in the ratio of heterophils/lymphocytes (Ebrahimzadeh et al. 2012). Furthermore, Zn supplementation can also improve antioxidant status. The Cu Zn-superoxide dismutase reduces free radicals by acting as a cofactor for Zn (Yu et al. 2020). Additionally, 45 mg/kg Zn sulphate in laying hens diet stimulates the formation of metallothionein,

which efficiently scavenges hydroxyl radicals (Niknia et al. 2022). The positive impact of dietary Zn on BWG and FCR may also be due to the increased intestinal absorptive surface area as indicated by the greater height and villi width in this research. The beneficial impact of Zn can increase the availability of nutrients and improve health, both of which lead to an increase in the ducks' BW.

Carcass

Zn supplementation effect on the carcass of the Sikumbang female Janti duck is in Table 4. Zn supplementation at 60 mg/kg (Z2 treatment) significantly (P<0.05) improved carcass weight in contrast to the control (Z0 treatment). Zn supplementation at 60 mg/kg significantly (P<0.05) reduced abdominal fat in contrast to Z0, and Z1 treatment. However, it was no different from the Z3 group (90 mg/kg). Carcass percentage was not impacted (P>0.05) by supplementation of Zn in the diet. However, the treatments did not show a significant effect (P>0.05) on the percentage of BW.

This research showed that the weight of the carcass increased with Zn supplementation in Sikumbang Janti female ducks. These results were consistent with research on turkey, which showed that adding 120-140 mg Zn/kg to the diet increased growth performance and carcass yield (Flores et al. 2021). This was due to the higher body weight of the ducks and the lower percentage of abdominal fat yielded by Zn supplementation treatment. Carcass weight is strongly influenced by live weight which in higher live weight results in greater carcass weight and *vice versa.*

This research's percentage of abdominal fat was reduced as Zn supplementation in the diet increased. According to Attia et al. (2013) and Hidayat et al.. (2020), dietary Zn supplementation decreased the proportion of abdominal fat compared to without Zn. According to other studies, variations in species and dietary Zn concentration could account for the lack of a rise in abdominal fat. Supplementation of 60 mg Zn/kg in a broilers diet could reduce abdominal fat by modulating lipogenic enzyme activity and gene expression, promoting hepatic fat metabolism, and stimulating lipid synthesis (Liu et al. 2015).

Digestive organs

Zn supplementation effect on the digestive organs of Sikumbang Janti female duck is shown in Table 4. Supplementation of Zn did not significantly affect (P>0.05) the digestive organs. These findings align with

Table 3. Effect of Zn supplementation in diets on productive performance and Zn consumption of Sikumbang Janti female duck aged from 8 to 16 weeks

Variables	Treatments				SEM	P-value
	Z0[1)]	Z1	Z2	Z3		
FC[2)] (g/bird)	5997.38	6047.09	5986.62	5967.22	26.86	0.79
BW (g/bird)	1314.[25a3)]	1417.62[b]	1420.83[b]	1400.16[ab]	17.74	0.09
BWG (g/bird)	599.08[a]	668.29[b]	668.46[b]	650.33[b]	15.83	0.02
FCR	10.81[a]	9.08[b]	8.99[b]	9.20[b]	0.26	0.02
Zn consumption (mg/bird)	177.46	360.35	536.34	713.62		

[1)]Z0 (Control diet), Z1 (Control diet+30 mg Zn/kg), Z2 (Control diet+60 mg Zn/kg), and Z3 (Control diet+90 mg Zn/kg), [2)]FC (feed consumption), BW (body weight), BWG (body weight gain), FCR (feed Conversion ratio), [3)]Means with different superscripts in the same row differ significantly (P<0.05)

Table 4. Effect of Zn supplementation in diet on carcass of Sikumbang Janti female duck aged from 8 to 16 weeks.

Variables	Treatments				SEM	P-value
	Z0[1)]	Z1	Z2	Z3		
Carcass weight (g)	710.25[a2)]	772.00 [ab]	800.250[b]	753.75[ab]	13.23	0.08
Carcass (%)	53.62	54.86	55.20	53.52	0.63	0.75
Abdominal fat (%)	1.58[a]	1.16[b]	0.86[c]	0.86[c]	0.08	0.00

[1)]Z0 (Control diet), Z1 (Control diet+30 mg Zn/kg), Z2 (Control diet+60 mg Zn/kg), and Z3 (Control diet+90 mg Zn/kg). [2)]Means with different superscripts in the same row differ significantly (P<0.05)

Table 5. Effect of Zn supplementation in diet on digestive and visceral organs of Sikumbang Janti female duck aged from 8 to 16 weeks

Variables	Treatments				SEM	P-value
	Z0[1)]	Z1	Z2	Z3		
Proventriculus (%)	0.42	0.41	0.37	0.38	0.01	0.48
Ventriculus (%)	3.14	2.92	2.96	2.88	0.08	0.71
Pancreas (%)	0.29	0.47	0.27	0.26	0.05	0.34
Liver (%)	2.61	2.16	2.45	2.38	0.09	0.40
Heart (%)	0.60	0.58	0.62	0.56	0.02	0.76
Spleen (%)	0.11	0.10	0.09	0.11	0.01	0.52
Bile (%)	0.12	0.12	0.10	0.10	0.01	0.49
Small Intestine (%)	2.92	2.92	2.99	3.39	0.11	0.44
Duodenum (cm)	26.33	27.50	27.66	28.87	0.47	0.32
Jejunum (cm)	69.66	72.33	72.50	70.00	1.07	0.73
Ileum (cm)	59.00	59.75	64.00	62.50	1.00	0.26
Ceca (%)	0.34	0.31	0.30	0.31	0.01	0.54
Colon (%)	0.28	0.29	0.24	0.29	0.01	0.17

[1)]Z0 (Control diet), Z1 (Control diet+30 mg Zn/kg), Z2 (Control diet+60 mg Zn/kg), and Z3 (Control diet+90 mg Zn/kg)

Table 6. Effect of Zn supplementation in diet on villus height (VH), villus width (VW), crypt depth (CD) and the ratio of villus height to crypt depth of Sikumbang Janti female duck aged from 8 to 16 weeks

Variables	Treatments				SEM	P-value
	Z0[1)]	Z1	Z2	Z3		
VH (mm)	0.69^{b}[2)]	0.72^{c}	0.79^{d}	0.64^{a}	0.01	0.001
VW (mm)	0.16^{a}	0.19^{a}	0.24^{b}	0.17^{a}	0.01	0.001
CD (mm)	0.24^{a}	0.20^{b}	0.17^{c}	0.18^{bc}	0.01	0.001
VH/CD ratio	2.86^{a}	3.47^{b}	4.67^{c}	3.43^{b}	0.08	0.001

[1)]Z0 (Control diet), Z1 (Control diet + 30 mg Zn/kg), Z2 (Control diet + 60 mg Zn/kg), and Z3 (Control diet + 90 mg Zn/kg). [2)]means with different superscripts in the same row differ significantly (P<0.01)

other earlier studies that found Zn supplements, both organic and inorganic, up to 120 mg/kg in the feed had no adverse effects on the visceral and digestive organ size (Attia et al.. 2013). Also, there was no difference in the percentages of pancreas, spleen, liver and heart when the ducks were fed with 30 mg/kg Zn oxide, but it increased the percentage of gizzard (Attia et al.. 2019). According to the previous study, adding 30-120 mg/kg (Ahmadi, 2013) and 30-160 mg/kg (Hidayat et al.. 2020) ZnO nanoparticles to broiler feed significantly affected liver weight. Several factors may explain the different effects of dietary Zn on internal organs, such as Zn concentration and source or type, rearing management, age, or environmental conditions.

Intestinal morphology

The impact of Zn Supplementation on villus height (VH), width height (WH), crypt depth (CD), and villus height/crypt depth (VH/DH) of Sikumbang Janti female duck is presented in Table 6. The Z2 treatment (60 mg Zn/kg) had the most significant (P<0.01) VH, VW, CD, VH/CD. These results align with the past result of Wu et al.. (2019) and Xie et al. (2021) that Zn supplementation improved the morphology of the small intestine as indicated by a decrease in the ratio of VH/CD, and an increase in duck VH.

In this research, an increase in VH and VW in the small intestine of Sikumbang Janti female ducks in Zn supplemented can be linked to the increased proliferation of crypt cell so that led to an increase in the digestive process and absorption due to an expansion of the nutrient absorption area, as indicated by better body weight gain and feed conversion efficiency. Our VH, CD, and VH/ CD ratio result were similar to those of Khajeh Bami et al.. (2018) who reported supplementation of 50 Zn-nano in a broiler diet were significantly better than a fed diet with 25 Zn-nano.

Similarly, Shah et al.. (2019) said that Zn supplemented 60 mg Zn/kg in a broiler diet improved VH, CD, and VH/CD ratio.

The digestion capacity and absorption of nutrients depend on the intestine's morphological conditions, especially the villi's surface area (Jia et al.. 2010). The elongated and larger villi show a greater surface area for nutrient absorption (Sacakli et al.. 2023). A smaller CD improved the intestine's capacity to absorb nutrients, A greater VH:CD ratio indicated enhanced digestion and absorption of nutrients in the small intestine (Shang et al.. 2020). Small intestines serve as a vital barrier against the introduction of toxic materials into the body, in addition to serving as a means of digestion and nutritional absorption.

Based on the results of this study, the use of Zn can be applied by farmers and the industry. The use of Zn minerals in the ration is small and low price, but the impact can improve performance so that the economic value also increases.

CONCLUSION

Dietary Zn at 60 mg/kg in Sikumbang Janti's diet increased BW, BWG and carcass weight and decreased FCR and abdominal fat without increasing feed intake, and the size of digestive organs. Supplementation of 60 mg Zn/kg) improved the greatest intestinal health, as indicated by an increase in VH, VW, CD, and VH/CD ratio.

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Morphological Characterization of Doe Kacang Goat in the Dry Land Area

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ABSTRAK

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Penelitian dilakukan untuk menyeleksi bibit sebagai populasi dasar berdasarkan karakter morfologi kambing Kacang betina dewasa di daerah lahan kering, menggunakan 31 ekor induk kambing Kacang. Sistem pemeliharaan semi intensif (tanpa pemberian konsentrat), pemberian air, hijauan (turi, gamal, dan lamtoro) dan rumput lapangan secara *ad libitum*. Deskripsi karakter kuantitatif dan indeks morfologi, korelasi pearson's bobot badan dengan ukuran dan indeks tubuh, dianalisis menggunakan program SPSS 25.0. Hasil analisis deskriptif BB, PB, TP, LiD, LD, DD, LPi, TPi, PPi, PKp, LKp, dan TKp berturut-turut adalah: 20.72±3.26 kg, 44.34±8.17 cm, 55.42±7.84 cm, 62.68±7.12 cm, 10.98±2.07 cm, 22.74±2.12 cm, 8.44±1.38 cm, 54.96±6.44 cm, 12.55±0.85 cm, 14.07±0.55 cm, 10.19±0.36 cm, dan 11.69±0.45 cm. Nilai indeks morfologi yakni WS, BI, DI, LI, PI, B, HS, FL, dan CI berturut-turut adalah 0.78+0.12, 71.06±12.38, 0.42±0.06, 0.81±0.18, 67.55±11.93, 0.43±0.08, 11.18±5.78, 32.68±7.40, dan 72.48±2.82. Kesimpulannya bahwa bobot badan dan ukuran tubuh induk kambing kacang pada sistem pemeliharaan semi intensif di daerah lahan kering masih dibawah standar mutu kambing Kacang Indonesia, koefisien korelasi bobot badan dengan ukuran tubuh berada pada kisaran positif sedang sampai tinggi ($P<0.05$) kecuali korelasi bobot badan dengan tinggi kepala menunjukan korelasi negatif. Koefisien korelasi bobot badan dengan indeks morfologi berada pada kisaran positif rendah kecuali koefisien korelasi bobot badan dengan width slope, balance dan heigth slope memiliki koefisien korelasi negatif.

Kata Kunci: Kambing Kacang Betina Dewasa, Lahan Kering, Indeks Morfologi

ABSTRACT

Beyleto VY, Hilmia N. 2023. Morphological characterization of doe Kacang Goat in the dry land area. JITV 28(2)143-151 DOI:http://dx.doi.org/10.14334/jitv.v28.i23234.

This study was conducted to select a breed based on the morphological characteristics of Kacang goats in the dry land area, by assessing 31 doe Kacang goats. Semi-intensive raising system is carried out (without giving concentrate), yet water; forage (such as *Gliricidia sepium leaves, Sesbania grandiflora leaves, Leucaena leucocephala leaves*) and local grass are given by applying *ad libitum* method. Description of morphological characteristics, Pearson's correlation between body weight and body size, and body index, were analyzed using SPSS 25.0 program. The result of descriptive analysis for BW, BL, WH, CG, CW, CD, RW, RH, RL, HL, HW, and HH are 20.72±3.26 kg, 44.34±8.17 cm, 55.42±7.84 cm, 62.68±7.12 cm, 10.98±2.07 cm, 22.74±2.12 cm, 8.44±1.38 cm, 54.96±6.44 cm, 12.55±0.85 cm, 14.07±0.55 cm, 10.19±0.36 cm, dan 11.69±0.45 cm respectively. The value of the morphological index for WS, BI, DI, LI, PI, B, HS, FL, and CI are 0.78+0.12, 71.06±12.38, 0.42±0.06, 0.81±0.18, 67.55±11.93, 0.43±0.08, 11.18±5.78, 32.68±7.40, dan 72.48±2.82, respectively. The conclusion is that the body weight and body measurements in the dry land area are still below the quality National standards of Indonesia. The correlation coefficient of body weight with body size is in the positive range of moderate to high except, the correlation of body weight with head height which shows a negative correlation. The correlation coefficient of body weight with the morphological index is in the low positive range except for the correlation coefficient of body weight with width slope, balance, and height slope which has a negative correlation coefficient.

Key Words: Doe Kacang Goat, Dry Land, Morphological Index

INTRODUCTION

Small ruminants (goats) have become farmers' choice because they have been part of local farming, especially in rural areas for quite a long time, easily adapt to climatic conditions (pasture conditions and other natural conditions), reproduce quickly, have high economic value, easy to raise, does not require large areas of land to raise, matures quickly, and is prolific (Rahadi et al. 2020; Suwignyo et al. 2018; Restitrisnani et al. 2013). Mashudi et al. (2022) reported that the land capacity of 2,014.87 can accommodate a goat population of 1,323,42 livestock units. Kacang goat is one of the genetic resources of local livestock in Indonesia,

including in Malaka Regency, East Nusa Tenggara Province. Kacang goat is mostly reared by farmerson a small scale (scale of ownership of 2-3 head of Kacang goat). This becomes a side business that is dominated by extensive and semi-intensive rearing systems and this business relies more on local potential. The world's goat breeding systems are dominated by extensive and semi-intensive systems with very low production costs (Escareño et al. 2013). Small and marginal farmers have very little capital, resources, and formal training (Ghosh et al. 2019). In Malaka, apart from Balinese cows and local pigs, Kacang goats can meet the needs of animal protein in overcoming the problem of stunting, as sacrificial animals in traditional rituals, and help farmers' finance, especially to pay their children's education costs of in Malaka Regency. Goats have a very significant contribution to rural communities, especially during crop failure. They are very adaptive and spread over various geographical conditions (Rawat et al. 2019; Berhe 2017).

Malaka Regency is a district whose government has the main work program, namely Malaka Agricultural Revolution (MAR), including the livestock sub-sector, namely the development of Kacang Goats. The goat population in Malaka Regency between 2019-2020 increased by 3.966 heads of the total increase in the goat population in East Nusa Tenggara Province during the same period was 164,116 heads (BPS-Statistics of Nusa Tenggara Timur Province 2021). The maintenance system in the Malaka district is dominated by an extensive and semi-intensive rearing system causing the productivity of goats to decrease due to inbreeding and negative selection. Population increase should be followed by improving the productivity of goats, especially the quality of livestock breeds. The morphological index can be used to determine the type and function of livestock so that it can help breeders to select livestock. The research on the morphological characteristics of the Kacang goat in the dry land area of Malaka Regency for selection purposes has never been carried out.

Characterization in livestock breeding systems is the first step in establishing policies for the development of the livestock sub-sector sustainably. This step is an alternative option and an important input for the efficiency of a breeding program design. This is also urgently needed in the development and conservation strategy and selection of productivity improvements. Further, this process can maintain livestock genetic resources and describes the type and phenotypic character of goats (Laouadi et al. 2018; Hosseini et al. 2016; Stojiljkovic et al. 2015; Mdladla et al. 2017; Pares Casanova, 2015). Furthermore, the assessment of variations in morphological traits is the first step in characterizing the genetic resources of local livestock (Rotimi et al. 2015). The characteristics of livestock breeds, namely size, and structure, especially livestock functions, are described through morphological index values (Dauda. 2018). One of the basic methods of classifying domestic goats based on origin, use, shape, and length of ears, is based on body size (Rotimi et al. 2017). Differences in climatic conditions between regions, adaptability, diversity of gene groups, natural selection, irregular mating systems, breeds, and livestock populations lead to differences in the characteristics of goat and sheep populations (Pares-Casanova. 2015). This study aims to breed select to form a basic population of Kacang goats in dryland areas based on the morphological characteristics.

MATERIALS AND METHODS

Goat population, location, and rearing system

A total of 31 doe Kacang goats aged 2-3 years, not pregnant, healthy, not disabled, has qualitative characteristic of black, white, brown, and mixed coat colors white, black, and brown, horns small and upright, ears small and erect side, straight and concave backlines, were used in this current study conducted at the Kacang goat breeding center in Naas Village, West Malaka District, Malaka Regency, East Nusa Tenggara Province, Indonesia. In this study, the rearing system was semi-intensive, adapting to the rearing patterns of local breeders. During the day, the goats are released for 8 hours in the pasture around the stables. The goats are not given concentrate, yet drinking water and forage are always available in the pen throughout the day (*ad libitum* method) in the form of *Gliricidia sepium leaves, Sesbania grandiflora leaves, Leucaena leucocephala leaves,* and field grass. The group pen is made of local materials, is divided into 6 plots measuring 4x5 m2, each plot containing five doe Kacang goats. Health control is carried out every 2 weeks and *B-com* vitamins are given once every 3 months.

Weight and size measurement

Goat's Body Weight (BW) was measured in kilograms (using a sitting scale with a capacity of 150 kg and an accuracy rate of 0.1 gr). Furthermore, linear body size was measured in centimeters (using measuring tape and calipers), i.e., measuring body Length (BL) according to Simmons & Ekarius (2009). Another way of measuring body size according to (Heriyadi et al. 2012) is Wither of Height (WH), Chest girth (HG), Chest Width (CW), Chest Deep (CD), Rump Width (RW), Rump Height (RH), and Rump Length (WL). Moreover, Head size Tagoi et al. (2020) is measured by calculating some aspects, namely Head Length (HL), Head Width (HW), and Head Height (HH). Kacang goat morphology index according to instructions (Salako. 2006; Pares-Casanova et al. 2013) is calculated by these formulas,

Table 1. The chemical composition of the research feed

Ingredients	Dry matter	Organic matter	Crude protein	Crude fat	Crude fiber	NNFE	Energy (Kkal/Kg)
Field grass[1]	32.50	91.49	9.74	3.67	29.66	48.40	3.06
Leucaena leucocephala[1]	27.50	93.44	26.85	7.41	13.90	42.57	3.39
Sesbania grandiflora[2]	27.87	91.50	27.37	11.48	7.30	52.90	4.378
Gliricidia sepium[3]			20.44	3.45	15.83		

Source: [1]Sanan (2018), [2]Tahuk et al. 2021), [3]Aminullah et al. (2022)

those are, Width slope (WS)= Hip Width (cm)/Chest Width (cm). Depth index (DI)= chest deep (cm)/Wither of Height (cm). Balance (B)= [waist length (cm) x hip width (cm)]/[chest deep (cm) x chest width (cm)]. Height slope (HS)= Wither of Height (cm) - waist height (cm). Pelvic index (PI)= (Rump width/ Rump length) x 100. Length index (LI)= body length (cm)/Wither of Height (cm), if the value of the positive length index is less than one then the livestock is included in the height type and if it is more than one then the livestock is included in the long type. Foreleg length (FL)= Wither of Height (cm) - chest deep (cm). Body Index (BI)= (Body length/ Chest girth) x 100, body index of goats can be grouped into three categories: long-line animals (BI>88); medigline animals (86<BI<88), and short or brevigline animals (BI<85). Cranial index (CI)= (Head width/head length) x 100.

Data analysis

Data analysis was carried out using descriptive analysis, namely the mean value, standard deviation, coefficient of variation (quantitative characters and morphological index), correlation of body weight with body size, and body index (analyzed using the Pearson correlation method) with the SPSS 25.0 program.

The variables of the correlation coefficient were computed as follows Sugiyono (2017):

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{(n\sum x^2 - (\sum x)^2)(n\sum y^2 - (\sum y)^2)}}$$

where r is the correlation coefficient of the variable x and variable y, n is number of sample, x is body weight and y is body size/body index.

RESULTS AND DISCUSSION

General description of the study area

Malaka Regency has an area of 1,160.63 km with conditions the morphology of most of the area hilly and mountainous with a degree of inclination (50%), temperatures ranging from 16.4-38.7, humidity 34-100%, average wind speed of 6.7 m/s, solar radiation 7.12%/month, low rainfall (16-69 mm/month), dominates the eastern region while high rainfall (120-172 mm/month) is found in most of the northern region (BPS-Statistic of Belu Regency 2020). The Kacang goat's rearing system is more dominated by traditional rearing system compared to semi-intensive and intensive rearing system. The purpose of raising Kacang goat is only a business side.

The location for the breeding of the Kacang goats is a new location formed as a center for breeding Kacang goats with a basic population of as many as 31 doe and 6 buck head, total area ± 1,500 m, located at Naas Village, West Malaka Sub District, Malaka District, Nusa Tenggara Province. At the breeding center, there is one pen unit in the form of group cages measuring 24x5 m, divided into 6 plots. Feed source comes from the pasture, agricultural, and forage wastes obtained around the breeding center location. During the rainy season, the temperature range in the study area is 20-29°C, and in the dry season 28-38°C.

Quantitative characters

The basic data for determining livestock rearing management and evaluating livestock development are body weight and body size. Quantitative characteristics of doe Kacang goats in this study are presented in Table 1. Body weight, wither of height, body length, and chest girth of the doe Kacang goats in this study did not meet the quality standards of the Kacang goat breeds according to the Decree of the Minister of Agriculture of the Republic of Indonesia No. 2840/Kpts/LB.430/8/2012, regarding the determination of the Kacang goat breed. It explains that body weight, body length, Wither of Height, and chest girth, are respectively 21.6±5.9 kg, 58.9±5.6 cm, 55.6±4.2 cm, and 63.2±7 cm. There is a difference in these average values because the standard setting for determining the breed of Kacang goats has gone through various stages and consideration of the commission for determining/releasing livestock breeds and strains (Heriyadi et al. 2012). Meanwhile, body weight, body length, chest girth, and wither of the height of the Kacang goats in this study were in the early stages of developing

Kacang goats with semi-intensive rearing management without giving concentrates.

In the semi-intensive rearing system, body weight, body length, Wither of Height , chest girth, chest width, chest deep, head length, rump height, and rump width of does Kacang goats respectively were 15.95±2.15 kg, 49.76±2.52 cm; 47.26±2.60 cm; 53.19±2.95 cm; 13.82±1.87 cm; 22.09±1.23 cm; 13.26±0.73 cm; 46.32±2.60 cm, and 15.92±1.74 cm (Azmidaryanti et al. 2017). Body weight, chest girth, chest deep, rump height, and head length (bigger) and the other body size lower from other studies on the same rearing pattern, sex, and age due to differences in rearing management (type and frequency of feeding), differences in adaptability to ecological conditions where rearing, and environmental conditions (temperature and humidity). Variations in livestock production performance are influenced by genetic and environmental factors in each region, such as feed, agro-climatic conditions, differences in management, selection systems, geographical location, and natural resources (Depison et al. 2020). In the dry land areas, ambient temperature and humidity range are between 23.67-28.23°C and 80.50-88.50%, respectively (Beyleto et al. 2022). High environmental temperatures have an impact on reducing feed consumption and increasing the frequency of drinking livestock, causing differences in the values of body weight and body size of livestock in different climatic conditions. The goats behavior to neutralize their hot body temperatures when they are in hot environmental temperatures includes lying in the shade, breathing with an open mouth, panting, reducing feed consumption, and increasing water consumption (Silanikove & Koluman. 2015; Mulyono et al. 2018). The response of livestock behavior in the production process has changed due to the influence of environmental changes (Rahmawati et al. 2022).

The coefficient value of body weight and body size variations of Kacang goats in this study was included in the medium category, which ranged from 10-20%. (Putra & Ilham 2019) stated that the coefficient of variation is in the moderate category if it ranges from >10% to <20%. The non-uniformity of the body weight and body size of the Kacang goats in this current study appeared because the goats come from several sub-districts around the location of the development of Kacang goats (Malaka Regency) with different initial rearing patterns and water availability. Some goats come from areas with abundant water availability and the provision of various types of feed, namely grass, gamal, waru, lamtoro, and agricultural waste (corn and peanuts). While, other goats come from areas with limited water availability and goats are released all day in pastures then kept in pens at night without feeding by breeders. Variations in ecological zones accompanied by unique climates and plant vegetation and have an impact on different management and environmental influences can cause morphological variations within and between goat populations (Birhanie et al. 2019).

The cranium size of Kacang goat in this study had a low level of variation, which was below 10%. The low value of the coefficient of diversity indicates that the head size of the Kacang goat at the study site is similar and has a close kinship relationship between individual livestock. Gomes & Valente (2016); Mahdi et al. (2013) stated that the craniometric approach is an effective method and does not require a large amount of money to determine the kinship of populations between and within an area, genetic distances, and population or individual characteristics.

Correlation of body weight with body size

One of the statistical values used to describe the degree of relationship between two variables is the correlation coefficient (Shirzeyli et al. 2013). The value of the correlation coefficient between body weight and body size of Kacang goats is described in Table 2.

The body weight of the Kacang goat has a positive correlation with all body measurements except head height. Body weight and body size of goats have a moderate to high positive correlation (Karna et al. 2020). The existence of mentioned correlation shows that these body measurements can be used to estimate body weight. Body size (body length, Wither of Height, and chest girth) can be used for individual selection, determining body weight, and describing frame size (Habib et al. 2019; Hankamo et al. 2020; Karna et al. 2020). Body weight with chest girth, chest width, and chest depth has the highest correlation coefficient values, namely 0.47, 0.66, and 0.60.

The correlation coefficient of body weight with body length, Wither of Height, chest girth, chest width, and hip length in Woyto-Guji goats was 0.84, 0.66, 0.85, 0.45, and 0.62 respectively (Zergaw et al. 2017). Depison et al. (2020) reported the correlation values of body weight with body length, Wither of Height, chest girth, chest depth, hip height, and hip width in Kacang goats in the lowland areas were 0.76, 0.79, 0.81, 0.75, 0.76, and 0.68, respectively. The correlation coefficient in this study differs from previous studies due to differences in the average value of body weight and body size. The differences in the correlation coefficient are due to differences in body weight and body measurement of Kacang goats.

Morphological index value of the Kacang goat

The body index method is very practical to use in the field because it is useful to describe the function and type of livestock based on body dimensions. In livestock scoring systems, the morphological index is an indicator

Table 1. Body weight and body size of Kacang goat

Variables	n	Mean±standard deviation	KV (%)
Body Weight (Kg)	31	20.72±3.26	15,75
Body Length (Cm)	31	44.34±8.17	18,42
Wither of Height (Cm)	31	55.42±7.84	14,15
Chest Girth (cm)	31	62.68±7.12	11,35
Chest Width (cm)	31	10.98±2.07	18,84
Chest Deep (cm)	31	22.74±2.12	19,31
Rump Width (cm)	31	8.44±1.38	16,41
Rump Height (cm)	31	54.96±6.44	11,72
Rump Length (cm)	31	12.55±0.85	16,77
Head Length (cm)	31	14.07±0.55	3.91
Head Width (cm)	31	10.19±0.36	3.53
Head Height (cm)	31	11.69±0.45	3.85

Table 2. Correlation coefficient of body weight and body size of Kacang goats

	BW	BL	SH	HG	CW	IC	HiW	WH	WL	HL	HW	HH
BW	1											
BL	0.42*	1										
SH	0.24	-0.17	1									
HG	0.47**	0.33	0.41*	1								
CW	0.66**	0.38*	0.24	0.46*	1							
IC	0.60**	0.43*	0.22	0.49**	0.72**	1						
HiW	0.32	0.21	0.13	0.30	0.55**	0.23	1					
WH	0.60**	0.43*	0.15	0.38*	0.53**	0.59**	0.28	1				
WL	0.38*	0.26	0.342	0.33	0.33	0.67**	-0.07	0.38*	1			
HL	0.31	-0.05	0.52**	0.15	0.11	-0.01	-0.02	0.11	0.17	1		
HW	0.40*	0.12	0.2566	0.22	0.25	-0.07	0.31	0.17	-0.11	0.47**	1	
HH	-.127	.094	0.13	0.20	0.12	-0.04	0.29	-0.13	0.04	0.09	0.31	1

*=P < 0.05; **=P < 0.01; BW=body weight; BL=body length; SH= Wither of Height ; HG=chest girth; CW=chest width; IC=chest deep; HiW=hip width; WH=waist height; WL=waist length; HL=head length; HW=head width; HH=head height

for determining the function and type of livestock and describes the relationship between various body sizes (Abdurrahman & Setiasih. 2017; Khargharia et al., 2015) The morphological index value of the Kacang goat is shown in Table 3.

An important parameter for estimating the function of a livestock breed is the width slope (Dauda. 2018). The width slope of the Kacang goat in this study was 0.78±0.12. Body index, length index, and depth index values for Kacang goats aged 2.5-3.5 years were 86.95±5.4, 1.07±0.09, and 0.46±0.04 respectively (Putra & Ilham. 2019). Body index, pelvic index, height slope, length index, and width slope of the local doe Ethiopian goat in the traditional rearing system were: 90.16±4.47,

68.53±8.56, 2.69±1.20, 92.41±4.86, and 1.04±0.12 (Getaneh et al. 2022). Pelvic index, balance, and height slope in Assam Hill goats were 92.49±2.54, 5.63±0.23, and 3.43±0.29 respectively (Khargharia et al. 2015). The cranial index value in Markhoz goats is 54.04±2.29 (Goodarz & Hoseini. 2014). Body index, pelvic index, length index, and foreleg index in local southern Ethiopian goats were: 87.5±0.28, 102±11.3, 1.03±0.04, and 18.8±4.38 (Dea et al. 2019). Differences in the morphological index values of Kacang goats in this study and other studies occur due to differences in body size and head size which become the components of the calculation of each morphological index value.

The pelvic index in this study is included in the linear-convex category because the result showed <100 where the hip length is greater than the hip width (Silva-Jarquin et al. 2019). It was further stated that based on baronian systematics, the body index values were longlinear (≥90), mesolinear (>86 and <88), and brevilinear (≤85). Based on baronian systematics, the body index of the Kacang goat obtained in this study was categorized into the brevilinear group (≤85) which shows that the width is smaller than the length.

The coefficient of variation in the morphological index of the Kacang goat is in the moderate category (Depth Index) to high (Width Slope, Body Index, length, balance, height slop, and Foreleg length) while the pelvic index and cranial index values are in a low category.

Correlation of body weight with the morphological index of Kacang goat

The correlation of body weight with morphological index is shown in Table 4. The correlation coefficient of body weight with length index, depth index, and body index in this study was higher than that of the Katjang goats in the Bone Balango Regency, respectively: 0.13, 0.01, and -0.25 (Putra & Ilham. 2019). It was further

Table 3. Morphology index of Kacang goat

Variable	n	Mean±standard deviation	KV(%)
Width Slope (WS)	31	0.78+0.12	15.03
Body Index (BI)	31	71.06±12.38	17.42
Depth Index (DI)	31	0.42±0.06	14.41
Length Index (LI)	31	0.81±0.18	22.71
Pelvic Index (PI)	31	67.55±11.93	3.88
Balance (B)	31	0.43±0.08	19.26
Height Slope (HS)	31	11.18±5.78	51.72
Foreleg Length (FL)	31	32.68±7.40	22.64
Cranial index (CI)	31	72.48±2.82	3.88

Table 4. Body weight correlation coefficient with Kacang goat morphological index values

	BW	BI	DI	LI	PI	WS	B	HS	FL	CI
BW	1									
BI	.19	1								
DI	.17	.43*	1							
LI	.19	.82**	.70**	1						
PI	.14	.02	-.05	.07	1					
WS	-.43*	-.23	-.28	-.18	.41*	1				
B	-.47**	-.25	-.35	-.25	.17	.93**	1			
HS	-.44	.13	-.06	.13	.10	.23	.30	1		
FL	.04	-.44*	-.93**	-.70**	.07	.12	.13	-.70*	1	
CI	.04	.12	.23	.27	.35	.05	-.05	.19	-.25	1

*=P<0.05; **=P<0.01;BW=body weight; BI=body index; DI=depth index; LI=length index; PI=pelvic index; Width slope; B=Balance; Height slope (HS); FL=Foreleg length; CI=Cranial index

stated that the correlation value between body weight and body index is included in the high category if it is $0.60<r<0.80$. From this explanation, the correlation value of body weight and body index in this study is involved in the low category, namely 0.19. The correlation of body weight with length index, depth index, and body index in Kacang goats in the lowland areas is 0.11, 0.58, and 0.20 respectively (Depison et al. 2020), -0.11, -0.33, and 0.47 in doe south African goats (Tyasi & Putra. 2021). The results showed that body weight with body index and length index had the highest correlation value (low positive) while body weight with width slope, balance, and height slope had negative correlation values in Kacang goats in dryland areas.

CONCLUSION

The body weight and body size of Kacang goats in dryland areas do not meet the quality standards of Indonesian Kacang goats. The value of the correlation coefficient of body weight and body size is in the low to high positive category except for the correlation coefficient of body weight and head height. The results showed that body measurement can be used to estimate the body weight of doe Kacang goat in dry land areas. The morphological index shows the characteristics of the Kacang goat, namely small and slender, while the value of the body weight correlation coefficient with the morphological index is in the negative to low positive range ($P<0.05$). For increase the value of morphological characteristics of Kacang goats in the dry land area, need to add concentrate in the feed.

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