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Jalan Raya Padjajaran Kav. E. 59, Bogor 16128 - Indonesia
Telephone (0251) 8322185
Fax (0251) 8380588
E-mail: criansci@indo.net.id; jitvnak@yahoo.com
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## PREFACE

In this edition, volume 24 No 2, we proudly present articles from animal and veterinary sciences including genetics; reproduction and food technology. The articles published in this edition are:

"Identification of GH|MspI and GHR|AluI Gene Polymorphism and its Association with Calf Birth Weight of Grati-PO Cattle"; "Genetic Polymorphism of SCD1 Gene of Holstein-Friesian Cows in Indonesia"; "Productivity of Bali Cattle Fed Ration Supplemented by Molasses Containing Several Types of Defaunation Agents"; "Enhance in-vitro rumen fermentation of *Panicum Maximum* with biological supplements"; "Traits of Sheep and Effects of Protein Supplements on Semen Profile in Indigenous Sheep of Bangladesh"; "Meat Quality on Sentul Cocks with Different Immunoglobulin Yolk Concentrations".

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 2019

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Acknowledgement

# Identification of GH|MspI and GHR|AluI Gene Polymorphism and its Association with Calf Birth Weight of Grati-PO Cattle

Hartati[1], Soewandi BDP[2], Hapsari AAR[2], Anwar S[3], Pamungkas D[1]

*[1]Beef Cattle Research Station, Jl. Pahlawan no. 2 Grati, Pasuruan, East Java 16784, Indonesia*
*[2]Indonesia Research Institute of Animal Production (IRIAP), Jl. Veteran III, Ciawi 16002, West Java, Indonesia*
*[3]Research Center for Biotechnology-Indonesian Institute of Sciences (LIPI), Jl. Raya Bogor Km. 46, Cibinong 16911, West Java – Indoensia*
*E-mail: hartati06@yahoo.com*

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**ABSTRAK**

Hartati, Soewandi BDP, Hapsari AAR, Anwar S, Pamungkas D. 2019. Identifikasi polimorfisme gen GH|MspI dan GHR|AluI dan hubungannya dengan bobot lahir pedet pada sapi Peranakan Ongole Grati di Loka Penelitian Sapi Potong. JITV 24(2): 55-61. DOI: http://dx.doi.org/10.14334.jitv.v24i2.1939

Bobot lahir pedet (BL) merupakan salah satu kriteria seleksi yang penting untuk memprediksi bobot badan dewasa dan kemudahan melahirkan pada sapi potong. Gen GH dan GHR dianggap sebagai kandidat gen yang bertanggung jawab atas sifat pertumbuhan pada sapi. Tujuan dari penelitian ini adalah untuk mengidentifikasi polimorfisme gen GH|MspI dan GHR|AluI dan dalam hubungannya dengan BL pada sapi PO Grati. Sebanyak 186 sapi PO Grati dipelihara di kandang percobaan Loka Penelitian Sapi Potong (Lolit Sapo) dari bulan Mei hingga Desember 2017. DNA genom diisolasi dari darah seluruh sampel sapi dan digunakan dalam analisis genotipe menggunakan metode PCR-RFLP. Hasil penelitian menunjukkan bahwa rata-rata BL sapi PO Grati dalam penelitian ini adalah 25,58 ± 3,31 kg. Tidak ada perbedaan statistik untuk BL pedet antara jantan dan betina. Frekuensi genotipe CC, CT dan TT pada gen GH masing-masing adalah 1,1, 18,8 dan 80,1%, dan frekuensi alel C dan T pada gen GH masing-masing adalah 0,105 dan 0,895. Sementara frekuensi genotipe AA, AG dan GG pada gen GHR masing-masing adalah 66,1, 25,3 dan 8,6%, dan frekuensi alel A dan G pada gen GHR masing-masing adalah 0,788 dan 0,212. Dapat disimpulkan bahwa baik gen GH|MspI dan GHR|AluI bersifat polimorfik tetapi tidak secara signifikan terkait dengan BL pedet pada sapi PO Grati.

**Kata Kunci**: Gen growth hormone, gen growth hormone receptor, sapi PO Grati, berat lahir pedet

**ABSTRACT**

Hartati, Soewandi BDP, Hapsari AAR, Anwar S, Pamungkas D. 2019. Identification of GH|MspI and GHR|AluI gene polymorphism and its Association with calf birth weight of Grati-PO Cattle. JITV 24(2): 55-61. DOI: http://dx.doi.org/10.14334.jitv.v24i2. 1939

Calf birth weight (CBW) is one of the important selection criteria to predict mature body weight and for calving ease in beef cattle. The GH and GHR genes are considered as candidate genes responsible for growth traits in cattle. The objectives of this study were to identify the polymorphism of GH|MspI and GHR|AluI genes and its association with CBW in Grati-PO cattle. A total of 186 Grati-PO cattle raised by Beef Cattle Research Station(BCRS) from May to December 2017. Genomic DNA were isolated from whole blood and used in genotyping analysis using the PCR-RFLP method. The result showed that the average of CBW of Grati-PO cattle in present study was 25.58±3.31 kg. There was no statistical difference for CBW between male and female. The genotype frequency of CC, CT, and TT of GH gene were 1.1, 18.8 and 80.1 %, respectively and allele frequency of C and T of the GH gene were 0.105 and 0.895, respectively. While the genotype frequency of AA, AG, and GG of GHR gene were 66.1, 25.3 and 8.6 %, respectively, and allele frequency of A and G of GHR gene were 0.788 and 0.212, respectively. It concluded that both GH|MspI and GHR|AluI gene are polymorphic but not significantly associated with CBW in Grati-PO cattle.

**Key Words**: Growth hormone gene, growth hormone receptor gene, Grati-PO cattle, calf birth weight

## INTRODUCTION

Ongole ascendant (PO) cattle or in Indonesian called as Peranakan Ongole (PO) is one of the most popular beef cattle breeds in Indonesia. This PO breed has contributed largely in the national fulfillment of beef meat in Indonesia. Therefore, genetic improvement has been conducted by Beef Cattle Research Station (BCRS), Ministry of Agriculture of Indonesia to produce superior breeding stock of PO cattle. The breed then called as Grati-PO cattle. However, up to present the selection is still conducted by conventional method based on phenotypic data to estimate the genetic value of Grati-PO cattle. The use of marker-assisted selection in breeding program is expected to accelerate the production of superior breeding stocks at BCRS.

Growth trait is one of economically importance traits which has major concern in beef cattle production.

Birth weight can be used as indicator to predict the future body weight of cattle because of directly related to growth rate and mature live weight (Biswas et al., 2003). Furthermore, moderate genetic correlation has been found between calf birth weight (CBW) with weaning and yearling weight in PO cattle (Hartati, 2016). Conversely, CBW is commonly used as major concern for calving difficulty or dystocia in herds (Johanson & Berger 2003; Gutierrez et al. 2007). The risk of dystocia increases with increasing in CBW (Gregory, Cundiff and Koch, 1995). However, Hartati (2016) showed that response to selection of CBW in PO cattle population is still low (4.8% pergeneration) and the selection results has not caused calving difficulties. Therefore, the selection of CBW still needs to be improved to get an optimal condition in Grati-PO cattle population.

Most of economic traits are quantitative traits and controlled by many genes which each contributes a small effect to the trait (Curi et al. 2006). The Somatotrophic axis plays a key role in controlling the regulation of metabolism and physiological proccess in mammalian (Renaville, Hammadi and Portelle, 2002). It essentially consists of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II) and their associated carrier proteins and receptors (Renaville, Hammadi and Portelle, 2002). Growth hormone is a main regulator for postnatal growth in mammals (Amiri et al. 2018). Whereas, growth hormone receptor (GHR) is a mediator of GH biological activity in target cells through stimulating myogenic signal transduction (Maskur & Arman 2014). Variation in GH and GHR has been found to be associated with growth traits in several breeds of cattle. GH|*Msp*I were found to be polymorphic and significantly associated with daily weight gain in PO cattle (Sutarno at al. 2005). Whereas, GHR gene becomes the genetic marker candidate and plays important role in GH and lactation process (Fontanesi *et al.*, 2007). The GHR gene in *Bos taurus* (Simmental and Limousin cattle) and *Bos javanicus* (Bali cattle) was known to be polymorphic (Zulkharnaim, Jakaria and Noor, 2010). Furthermore, GHR gene polymorphism has been studied and has the effect on final weight and carcass traits in *Bos taurus* (Han et al. 2009).

According to previous study, the two genes (GH and GHR) could be used as strong candidate genetic marker for growth traits in cattle. These two genes could be used to support genetic selection in Grati-PO cattle. There was no report on the association of GH and GHR genes polymorphism and CBW in PO cattle. Thus, the objectives of this study were to identify the polymorphism of GH|*Msp*I and GHR|*Alu*I genes and its association with CBW in Grati-PO cattle.

## MATERIALS AND METHODS

### Animals and DNA

Data records of the calves birth weight were collected from 186 Grati-PO cattle raised by BCRS. Data records were collected from May to December 2017. Blood samples were collected from jugular vein into 3 mL vaccutainer tubes containing K3EDTA. DNA were isolated from whole blood samples using DNA extraction kit (Qiagen, Taiwan) and then stored -20°C for further use.

### PCR amplification and PCR-RFLP

The specific fragments containing SNPs of GH and GHR gene were amplified using primer pairs designed by Sutarno et al. (2005) and Di Stasio et al. (2005), respectively. The primer information used is given in Table 1. PCR reaction was performed in a total volume of 10 µL containing of approximately 10 ng/µL of DNA, 0.2 µM of each primers, 5 µL of MyTaqTM HS Red Mix (Bioline, USA), and dd$H_2O$ to a final volume

**Table 1.** The primers used to amplify specific fragments of GH and GHR gene in Grati-PO cattle

| | Locus | |
|---|---|---|
| | GH\|*Msp*I | GHR\|*Alu*I |
| SNPs Position | +837C/T | 257A/G |
| Region | Intron 3 | Exon 10 |
| GenBank Accession no. | JQ711182.1 | AF140284.1 |
| Primer Sequences (5' to 3') | F : CCCACGGGCAAGAATGAGGC | F : GCTAACTTCATCGTGGACAAC |
| | R : TGAGGAACTGCAGGGGCCCA | R : CTATGGCATGATTTTGTTCAG |
| Amplicon Size (bp) | 329 | 342 |
| Annealing temp (°C) | 65.7 | 53.8 |
| References | Sutarno et al. (2005) | Di Stasio et al. (2005) |

of 10 µL. The PCR conditions were pre-denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing for 15 s at 65.7°C (for GH|*Msp*I) and 53.8°C (for GHR|*Alu*I), extension at 72°C for 10 s, and a final extension at 72°C for 5 min. PCR products were electrophoresed on 1% agarose gels, stained with GelRed®10,000X in water (Biotium, USA) and visualized under a G-BOX Gel Documetation System (Syngene, UK). The PCR products of GH|*Msp*I and GHR|*Alu*I were digested with *Msp*I and *Alu*I restriction enzyme, respectively (New England Biolabs, USA). The digested fragments were electrophorezed on 3% agarose gels, stained with GelRed®10,000X in water (Biotium, USA) and visualized under a G-BOX Gel Documetation System (Syngene, UK).

**Statistical Analysis**

Genotypic and allelic frequencies were calculated by direct counting. Deviation from Hardy–Weinberg equilibrium (HWE) were analyzed using a Chi-square test. Population genetic indexes including observed heterozygosity (Ho), expected heterozygosity (He) were calculated based on Allendorf & Luiart (2007), and value of PIC was calculated based on Botstein et al. (1980). Association between genotypes and CBW were analyzed using GLM model by SPSS IBM version 20.0 software.

## RESULTS AND DISCUSSION

**Calf Birth Weight of Grati-PO cattle**

The descriptive statistic of CBW in Grati-PO cattle is given in Table 2. The average of CBW of Grati-PO cattle in present study was 25.58±3.31 kg. This CBW was not different from previous study using collection data from 2004 to 2013 (22.3±3.0 to 25.8±3.3 kg) (Hartati, 2016), as well as from PO cattle in Gunung Kidul district, special region of Yogyakarta (26 to 28 kg) (Baliarti, 1991), but these findings are higher than in Sumba Ongole cattle (SO) (21.20±4.60kg) (Said et al. 2016). Furthermore, CBW of Grati-PO cattle were superior than in other local Indonesian cattle such as Bali (17.73±1.72 kg) (Gunawan & Jakaria, 2011), Madura (19.78±1.22 kg) (Kutsiyah et al. 2003) or Aceh cattle (12.77±0.76) (Putra et al. 2016). However, CBW of Grati-PO cattle was much lower than in Kebumen-PO cattle (up to 31.88 ± 3.78 kg) (Maharani et al. 2018) or from the same *Bos indicus* cattle breeds in other countries such as Brahman in South Africa (32 kg) (Schoeman 1996), Brahman in Columbia (33.06±3.60kg) (Martínez et al. 2017), and Nellore in Brazil (32.30±3.80 kg) (Chud et al. 2014). This recent findings showed that breeds of cattle was associated with CBW and Ongole cattle breeds have a potential to produce higher CBW than other Indonesian local cattle. Nevertheless, CBW of Ongole cattle breed including Grati- PO cattle were much lower than in *Bos taurus* cattle breeds such as in Charolais (41 kg), Limousin (38 kg) (Schoeman, 1996), Friesian Holstein (33.8±0.6 kg) (Dhakal et al. 2013) and Belgian Blue cattle (49.2±7.1 kg) (Kolkman et al. 2010).

Based on statistical analysis, there was no differences of CBW of Grati-PO cattle between male and female ($p>0.05$) in present study, however CBW in male (26.08 ± 3.02 kg) tended to higher than in female (25.14±3.02 kg). In general, CBW in male and female were significantly different such observed in several previous study both in local or exotic breeds of cattle (Van Vleck & Cundiff 1998; Raphaka & Dzama 2009; Casas et al. 2012; Prasojo et al. 2010; Dillon et al. 2015; Hartati 2016; Said et al. 2016). Difference between males and females was found about 2.3 kg by Casas et al. (2012), 4.4 kg more by Herring et al. (1996). The average weight of males are being heavier than females in some stage of growth (Raphaka & Dzama 2009; Casas et al. 2012) Therefore, sex should be used as adjustment or correction factor in cattle genetic evaluation for a fair comparison of animals (Bayou et al. 2015; Raphaka & Dzama 2009). Beside sex, sire breed (Casas et al. 2012), weight of dam, season and year of calving are several factors that should be considered because affecting on CBW.

**Table 2**. Descriptive statistics of the birth weight calves performance of male and female of Grati-PO cattle

| Sex | n | Mean±SD (kg) | Min (kg) | Max (kg) | CV (%) |
|---|---|---|---|---|---|
| Male | 87 | 26.08±3.56 | 20 | 40 | 13.64 |
| Female | 99 | 25.14±3.02 | 16 | 35 | 12.03 |
| Total | 186 | 25.58±3.31 | 16 | 40 | 12.94 |

n = number of samples; SD= standard deviation; CV= coefficient of variation; Min = minimum value; Max = maximum value

The evidence of average of CBW in present study that was not different from previous studies (Hartati, 2016) indicates that the response to selection of CBW in Grati-PO cattle may still low. Therefore, the selection of CBW still needs to be improved. Marker-assisted selection technology could be used to accelerate genetic improvement of CBW in Grati-PO cattle. However, the optimum value of CBW should be considered because it affects calving difficulties that increases risk of death in cows and calves and additional veterinary cost (Johanson & Berger 2003; Zaborski et al. 2009).

### *GH*|*Msp*I and *GHR*|*Alu*I gene polymorphism

The allele and genotype frequencies of *GH*|*Msp*I and *GHR*|*Alu*I in Grati-PO cattle are shown in the Table 3 and Table 4. In *GH*|*Msp*I gene, the TT genotype (or *Msp*$I^{-/-}$ genotype) was the most frequent genotype (80.1%) observed in Grati-PO cattle, while the CC genotype (or *Msp*$I^{+/+}$ genotype) found to be rare (1.1%). In previous study, the *Msp*$I^{-/-}$ genotype was also the most frequent genotype (79%) in Indian Ongole (Sodhi et al. 2007). Although the frequency is lower than the present study, Musa et al. (2013) also reported the same results that the *Msp*$I^{-/-}$ genotype was the highest genotype found in Kenana (67%) and Butana cattle (47%). In *Bos taurus* cattle, the *Msp*$I^{+/+}$ genotype relatively high such in Holstein heifers (77%) (Arango, et al. 2014), Limousin (40.9%) and Simmental (77.3%) (Jakaria et al. 2009). The *Msp*$I^{-}$ allele was the common allele found Grati-PO cattle (0.895). This pattern is also

found in other Indonesian breeds of cattle such in Pesisir cattle (0.800) (Jakaria et al. 2007), Bali cattle (1.000) (Jakaria et al. 2009) and Sumba Ongole cattle (0.820) (Agung et al. 2018). Interestingly, this is contrary to the result on PO cattle from Grobogan district (Grobogan-PO cattle), where the frequency of *Msp*$I^{-}$ allele was lower (0.26) than *Msp*$I^{+}$ allele (0.76) and the *Msp*$I^{-/-}$ was the lowest genotype (Sutarno et al. 2005). Meanwhile, the frequency of C allele was found to be high in *Bos taurus* cattle such in Limousin (0.636) and Simmental cattle (0.889) (Jakaria et al. 2009). This result indicates that the *Msp*$I^{-/-}$ genotype and *Msp*$I^{-}$ allele may be high in *Bos indicus* since it was also found to be highest genotype and allele in 17 Indian cattle breeds (Bos indicus) (Sodhi et al. 2007) and as explained by Lagziel et al. (2000). The difference genotype and allele frequency in Grobogan-PO cattle from other *Bos indicus* cattle may be due to the different of selection and breeding history. However, it needs further investigation

In *GHR*|*Alu*I, the AA genotype was observed to be the most frequent (66.1%), while the GG genotype found in lowest proportion (8.6%) in Grati-PO cattle. The A allele was the common allele found in Grati-PO cattle (0.788). In other Indonesian local cattle, AA genotype was also as the common allele such in Bali cattle (99.8%) and Pesisir cattle (60.4%) as well as for

**Table 3**. Allele and genotype frequencies of GH|*Msp*I in Grati-PO cattle

| Gene | Sex | n | Genotype Frequency (%) | | | Allele Frequency | | χ2test |
|---|---|---|---|---|---|---|---|---|
| | | | *Msp*$I^{+/+}$ | *Msp*$I^{+/-}$ | *Msp*$I^{-/-}$ | *Msp*$I^{+}$ | *Msp*$I^{-}$ | |
| GH|*Msp*I | Male | 87 | 1.1 (1) | 23.0 (20) | 75.9 (66) | 0.126 | 0.874 | 0.144 |
| | Female | 99 | 1.0 (1) | 15.2 (15) | 83.8 (83) | 0.086 | 0.914 | 0.120 |
| | Total | 186 | 1.1 (2) | 18.8 (35) | 80.1 (149) | 0.105 | 0.895 | 0.001 |

n = number of samples; χ2tab = 3.841, χ2test <χ2tab means the genotype frequency is in HWE

**Table 4**. Allele and genotype frequencies of GHR|*Alu*I in Grati-PO cattle

| Gene | Sex | n | Genotype Frequency (%) | | | Allele Frequency | | χ2test |
|---|---|---|---|---|---|---|---|---|
| | | | AA | AG | GG | A | G | |
| GHR|*Alu*I | Male | 87 | 65.5 (57) | 26.4 (23) | 8.0 (7) | 0.787 | 0.213 | 3.855 |
| | Female | 99 | 66.7 (66) | 24.2 (24) | 9.1 (9) | 0.788 | 0.212 | 7.472 |
| | Total | 186 | 66.1 (123) | 25.3 (47) | 8.6 (16) | 0.788 | 0.212 | 11.133 |

n = number of samples; χ2test <χ2tab means the genotype frequency is in HWE

a allele in Bali cattle (0.991) and Pesisir cattle (0.615) (Zulkharnaim et al. 2010). Same as in Simmental cattle, the most frequent genotype was AA genotype (64.19%) and the A allele was the common allele (0.720) (Ardicli et al. 2017) However, it differs from Simmental and Limousin raised in Malang Artificial Insemination Center (BBIB Singosari), Malang which the G allele was the common allele (0.714 and 0.735, respectively (Zulkharnaim et al. 2010). While, Di Stasio et al. (2005) have identified the A and G allele were almost equally distributed in Piemontese cattle.

**Gene association**

Analysis results for the association between genotype of *GH|Msp*I and *GHR|Alu*I with CBW in Grati-PO cattle is shown in Table 5. In the present study, genotype of *GH|Msp*I gene was not significantly associated with CBW in Grati-PO cattle either using separated analysis between males and females or combined from two sexes. No significant differences between genotype and mature body weight (MBW) was also observed in Pesisir cattle (Jakaria et al. 2007). However, GH|*Msp*I gene was found to be associated with MBW in Grati dairy cows, in which CC genotype were found to be higher than CT and TT (Maylinda 2011). Different results were found by Arango, et al. (2014), in which the TT genotype was the highest for parameters of weight at first estrus and weight at first calving ($p<0.05$) in Holstein heifers. These indicate that the *GH|Msp*I gene has a different effect on body weight at different stage of the physiological status and cattle breeds. However, Arango et al. (2014) stated that the T allele is favorable in cattle specialized for meat production while the C allele is favourable in cattle specialized for milk production. Grati-PO cattle is meat-type breed of cattle, and in the present study gave indication that TT genotype tend to be higher in CBW than CT genotype, although it is not statistically different.

In *GHR|Alu*I gene, the genotype was also not significantly associated with CBW in Grati-PO cattle. This result is similar to those reported by Di Stasio et al. (2005) and Ardicli et al. (2017), for association between *GHR|Alu*I gene and growth traits in Piemontese, Angus and Simmental cattle. However, Di Stasio et al. (2005) showed that the A allele is significatly higher in drip loss than the G allele in Piemontese and Angus cattle. In other study, (Komisarek et al. 2011) reported that *GHR|Alu*I gene affected milk fat and protein content on 209 individuals of Jersey cattle. It gave some indication that the *GHR|Alu*I gene might do not associated with growth and production traits, but associated with meat and milk quality traits.

**Table 5**. Association between genotype and CBW in Grati-PO cattle

| Sex | GH|*Msp*I[a] | | | | GHR|*Alu*I | | | |
|---|---|---|---|---|---|---|---|---|
| | Genotype | n | CBW (kg) | Sig. | Genotype | n | CBW (kg) | Sig. |
| Male | +/+ | 1 | 30.5 | - | AA | 57 | 25.8±3.4 | ns |
| | +/- | 20 | 25.7±2.9 | ns | GA | 23 | 26.1±3.1 | ns |
| | -/- | 66 | 26.1±3.7 | ns | GG | 7 | 28.1±5.5 | ns |
| Female | +/+ | 1 | 30.0 | - | AA | 66 | 25.3±3.3 | ns |
| | +/- | 15 | 24.4±2.8 | ns | GA | 24 | 24.7±2.7 | ns |
| | -/- | 83 | 25.2±3.0 | ns | GG | 9 | 25.3±1.3 | ns |
| Male and Female | +/+ | 2 | 30.3±0.4 | - | AA | 123 | 25.5±3.4 | ns |
| | +/- | 35 | 25.1±2.9 | ns | GA | 47 | 25.4±2.9 | ns |
| | -/- | 149 | 25.6±3.4 | ns | GG | 16 | 26.6±3.9 | ns |

n = number of samples
CBW = calf birth weight
[a]both in male, female and its combination, the frequency of *Msp*I$^{+/+}$ genotype is very small, so this record was not included in analysis
sig. = significance level of 0.05
ns = not significant

## CONCLUSION

It could be concluded that both *GH*|*Msp*I and *GHR*|*Alu*I gene are polymorphic but not significantly associated with CBW in Grati-PO cattle. Further investigation in larger samples and other cattle breeds will needed to study the effect of the *GH*|*Msp*I and *GHR*|*Alu*I on CBW.

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# Genetic Polymorphism of SCD1 Gene of Holstein-Friesian Cows in Indonesia

Wulandari AS[1], Rahayu HD[2], Volkandari SD[1], Herlina N[1], Anwar S[1], Irnidayanti Y[2]

[1]Research Center For Biotechnology LIPI, Jalan Raya Bogor Km.46, Cibinong, Bogor 16911
[2]Biology Programme, Departmen Of Mathematics And Natural Science, State University Of Jakarta.
Jl. Pemuda No. 10 Rawamangun, Jakarta Timur
E-Mail: Ari.Wulandari5487@gmail.Com

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ABSTRAK

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Stearoyl-Coenzyme A Desaturase 1 (SCD1) tergolong sebagai famili dari asam lemak jenuh. Pada ternak ruminansia yang menghasilkan susu, protein SCD1 sering kali diekspresikan pada kelenjar menyusui dan relevan berpengaruh pada komposisi susu dan produk susu lainnya. Polimorfisme gen SCD1 pada sapi Friesian Holstein (FH) dapat digunakan sebagai dasar seleksi ternak secara molekuler untuk meningkatkan produktivitas. Tujuan dari studi ini adalah untuk mengetahui polimorfisme gen SCD1 pada sapi Friesian Holstein yang ada di Indonesia. Sebanyak 162 ekor sapi FH yang berasal dari 4 lokasi (Bogor, Sukabumi, Tasikmalaya dan Enrekang) digunakan dalam penelitian ini. Genotyping gen SCD1 menggunakan metode PCR-RFLP dengan enzim restriksi NcoI. Hasil menunjukkan bahwa tiga genotip (AA, AV dan VV) dan dua alel (A dan V) telah berhasil ditemukan dan bersifat polimorfik. Alel A ditemukan dominan (0.63) pada seluruh sampel dan dalam keseimbangan genetik. Frekuensi alel A tertinggi ditemukan di lokasi Sukabumi (0.78) sedangkan yang terendah di Bogor (0.55). Nilai heterosigotitas teramati (Ho) dan harapan (He) sebesar 0,471 dan 0,470. Kesimpulan, polimorfisme ditemukan pada semua lokasi dengan alel A sebagai alel dominan. Hasil ini dapat menjadi informasi genetik awal sapi FH di Indonesia dan untuk membentuk strategi *breeding* sapi perah agar dapat meningkatkan produktivitas khususnya peningkatan lemak susu sehat.

**Kata Kunci**: Sapi Perah Friesian Holstein, SCD1, Polimorfisme

**ABSTRACT**

Wulandari AS, Rahayu HD, Volkandari SD, Herlina N, Anwar S, Irnidayanti Y. 2019. Genetic polymorphism of SCD1 gene of Holstein-Friesian cows in Indonesia. JITV 24(2): 70-75. DOI: http://dx.doi.org/10.14334.jitv.v24i2.1905

Stearoyl-Coenzyme A desaturase 1 (SCD1) belongs to the fatty acid family of desaturases. In lactating ruminants, the SCD1 protein is highly expressed in the mammary gland and is relevant for the fatty acid composition of milk and dairy products. Polymorphism of SCD1 gene in Holstein-Friesian (HF) cows could be used as a basis of molecular selection of cattle in order to increase their productivity. The aim of this study was to investigate the polymorphism of SCD1 gene of Holstein-Friesian cows in Indonesia. A total of 162 blood samples of HF cows were collected from four different locations i.e. Bogor, Sukabumi, Tasikmalaya and Enrekang districts. Genotyping of SCD1 gene used PCR-RFLP method with NcoI restriction enzyme. The result showed that three genotypes (AA, AV and VV) and two alleles (A and V) have successfully found and polymorphic. A allele was dominant in all populations (0.63) and in Hardy Weinberg Equilibrium. The highest A allele was found in Sukabumi (0.78) and the lowest was in Bogor (0.55). Heterozigosity observed and expected reached 0.471 and 0.470, respectively. In conclusion, genetic polymorphism was found in all population with dominant of A allele. This finding can be used as a early genetic information of Holstein-Friesian cattle in Indonesia and to build breeding strategy for improving of productivity especially improving of healthy fat milk.

**Key Words**: HF Dairy Cow, SCD1, Polymorphisms

## INTRODUCTION

The gene encoding Steroyl-Coenzyme A Desaturase 1 (SCD) was mapped to bovine chromosome 26 (Campbell et al. 2001), where some QTLs for fat yield and other milk traits have been also identified. Based on Genome Wide Association Study in Dutch Dairy cattle, fat content in milk was influenced from several genes and one of them was SCD1 in BTA26 (Bouwman et al. 2011).

Considerable amount of studies have shown significant role of genotypes SCD1A293V in exon 5 toward the configuration of fatty acids in milk and milk production traits (Taniguchi et al. 2004; Moioli et al. 2007; Kgwatalala et al. 2009; Clark et al. 2010; Kulig et al. 2016). Hence, traits selection through different type of genotype can be beneficial to change the composition of milk fat. A high amount of saturated fatty acids and a low amount of (poly) unsaturated fatty acids contribute to milk traits. So, essential to human health, the upsurge

in unsaturated fatty acid amount in milk has become important objective in some studies (Taniguchi et al. 2004; Schennink et al. 2008; Kgwatalala et al. 2009; Mashhadi et al. 2012).

SCD is a key enzyme responsible in desaturation of fatty acid in mammary gland and others tissues. Stearoyl-CoA desaturase (SCD) plays an important role in bovine mammary lipid metabolism as it introduces a cis-double bond in the D9 position of a wide range of fatty acids (FA). It has a substrate preference of C18:0 and to a lesser extent C16:0, which are converted into C18:1 cis-9 and C16:1 cis-9, respectively (Ntambi 2004). C18:1 cis-9 has a far lower melting point than C18:0. Therefore, the SCD plays a vital role in keeping the fluidity of cell membrane and milk fat. The fatty-acid (FA) fluidity was determined by the length and quantity of FA chains included in the orientation of its paired-chains. Since the FA profile influence milk fluidity, it is used in triglyceride synthesis of milk fat. Milk fluidity can be reduced by decreasing the number of both FA's short and medium-chains. This occurs because of inhibition of the synthesis of FA mammary de novo (Maxin et al. 2011; Jacobs et al. 2013). SCD is also responsible for the conversion of C18:1 trans-11 to C18:2 cis-9, trans-11, which has been perceived related to several medical benefits towards human, one of them is anti-carsinogenic and anti-atherogenic effect (Bhattacharya A, Banu J, Rahman M 2006; Reynolds CM and Roche HM 2010).

The objective of this study is to identify the possibility of polymorphism in SCD gene using PCR-RFLP technique. The study also investigates the correlation of SCD gene polymorphism to milk fat production. The finding of this study is substantially useful in a trait selection design of dairy cattles which will improve of milk fatty-acid productivity. In practice, the selected dairy cattles from this design can be delivered either to small farmers or industries and trigger improvement in milk production.

## MATERIALS AND METHODS

### DNA Samples

A total of 162 fresh blood samples of Holstein-Friesian dairy cows were used as DNA resources for genotyping of SCD1 gene. Three milliliters of blood of each individual cow were collected into vacutainer tube containing $K_3$EDTA via jugular vein. All of samples were taken from four districts i.e. Enrekang, Tasikmalaya, Sukabumi and Bogor (Table 1.) Genomic DNA isolation was performed using Genomic DNA mini kit (Geneaid Biotech Ltd., Taiwan) by following to the the manufacturer's instructions. The collected DNA was stored at -20°C for further use.

**Table 1.** Details of the number samples of Holstein-Friesian dairy cows in Indonesia used for genotyping of SCD1 genes

| Provinces | Districts | Number of animals |
|---|---|---|
| South Sulawesi | Enrekang | 45 |
| West Java | Tasikmalaya | 19 |
| West Java | Sukabumi | 23 |
| West Java | Bogor | 75 |
| Total | | 162 |

### Genotyping

Genotyping of SCD1 gene was conducted by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. A 400 bp of specific fragment was amplified using a pair primer of forward: 5-CCCATTCGCTCTTGTTCTGT-3 and reverse 5-CGTGGTCTTGCTGTGGACT-3 (Kgwatalala et al. 2009). The PCR was performed in a 10 µl reaction mixture containing 5 µl PCR Master Mix (My Taq HS Red mix Bioline, USA), 1 µl each of primer (10pmol/µl), 2 µl water free nucleases and 1 µl DNA template. The PCR mixture was run in a thermal cycler machine (Eppendorft, Germany) following program pre-denaturation 94$^o$C for 5 minutes, 35 cycles of denaturation at 94$^o$C for 45 seconds, annealing 56.7$^o$C for 30 seconds, extension 72$^o$C for 45 seconds and final extension 72$^o$C for 5 minutes. PCR products were checked using 1% agarose gel (100 V, 60 minutes) and visualized under UV light by UV Transilluminator (Major Science, USA).

RFLP method was performed using *Nco*I enzyme (Kgwatalala et al. 2009). The cutting site of *Nco*I enzyme is 5'-C*CATGG-3'. The PCR product were digested in a total volume 10 µl containing 5 µl PCR product, 0.3 µl NCoI restriction enzyme (10U/µl), 1 µl reaction 10x buffer and 3.7 µl ddH2O. The mixture was then incubated at 37$^o$C for 3 hours and inactivated at temperature of 65$^o$C for 20 minutes. The digested PCR products were electrophoresed using 2.5% agarose gel for an hour of 100 Voltage. The fragments were stained using GelRed for an hour and visualized under UV light by UV Transiluminator (Major Science, USA).

### Data analysis

Genotypes and allele frequencies, heterozygosity observed (Ho), heterozygosity expected (He) and Hardy Weinberg Equilibrium (HWE) was direct calculated by Nei dan Kumar (Nei 2000).

## RESULTS AND DISCUSSION

A 400 bp targeted-fragment of the SCD1 gene were successfully amplified using Polymerase Chains Reaction (PCR) technique in all samples (Figure 1). Mutation at position 10329 caused changing of Cytosine to Thymine that influenced of amino acid Alanine (A) to Valin (V). The mutation was known as SNP A293V and recognized by *Nco*I restriction enzyme (5'-C*CATGG-3') (Taniguchi et al., 2004). SCD1|*Nco*I restriction analysis revealed that there were three genotypic patterns and two alleles (A and V) in all of Holstein-Friesian dairy cow population. This finding is similar as described by (Taniguchi et al. 2004; Mele et al. 2007; Milannesi et al. 2008). Those three patterns were single fragment of 200 bp (referred to AA genotype), single fragment of 400 bp (VV genotype) and two fragments of 200 bp and 400 bp were a heterozygous of AV genotype (Figure 2).

The genotype and allele frequencies of SCD1 gene for HF dairy cow in all population were presented in Table 3. The frequency of AA genotype of FH dairy cow in Sukabumi (0.61) was higher than those in Tasikmalaya (0.42), Enrekang (0.36) and Bogor (0.23). Frequency of the AV genotype in Bogor population (0.65) was higher than those in Enrekang (0.58), Tasikmalaya (0.42) and Sukabumi (0.35). The results showed that those FH dairy cows with the VV genotype were low in all population (Enrekang = 0.06; Tasikmalaya = 0.16; Sukabumi = 0.04; Bogor = 0.12). The allele frequencies for HF dairy cows in all population were under HWE condition, indicating random mating with respect to this locus. Gene equilibrium in population is achieved when selection, mutation, migration and genetic drift are absent (Falconer and T.F.C. Makay 1996). Average value of heterozigosity observed (Ho) reached 0.471 while heterozigosity expected was 0.470. Those values refected of genetic diversity of SCDI gene in four populations.

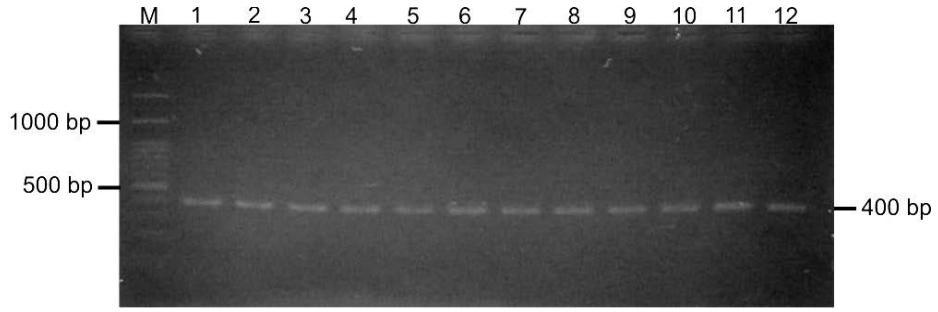


**Figure 1**. Visualization of PCR product of SCD1 gene from HF dairy cow samples (1-12); M = 100 bp DNA ladder

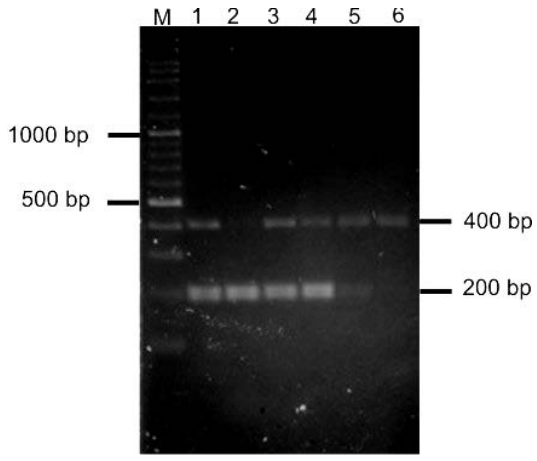


**Figure 2**. Visualization of PCR-RFLP products of SCD1 gene.
M = 100 bp DNA ladder
Line 1,3-5 = AV genotypes
Line 2 = AA genotype
Line 6 = VV genotype

**Table 2.** Allelic and Genotypic Frequencies, heterozigosity and HWE value of SCD1 Gene in Friesian Holstein

| Population | N | Allelic Frequency | | Genotypic Allelic | | | Ho | He | HWE | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | V | AA | AV | VV | | | ($\chi$2value) | ($\chi$2tab) |
| Enrekang | 45 | 0.64 | 0.36 | 0.36 | 0.58 | 0.06 | 0.463 | 0.458 | 3.060 | 3.841 |
| Tasikmalaya | 19 | 0.63 | 0.37 | 0.42 | 0.42 | 0.16 | 0.478 | 0.465 | 0.172 | 3.841 |
| Sukabumi | 23 | 0.78 | 0.22 | 0.61 | 0.35 | 0.04 | 0.348 | 0.340 | 0.011 | 3.841 |
| Bogor | 75 | 0.55 | 0.45 | 0.23 | 0.65 | 0.12 | 0.498 | 0.494 | 7.762 | 3.841 |
| Total | 162 | 0.62 | 0.38 | 0.34 | 0.56 | 0.10 | 0.472 | 0.471 | 6.008 | 3.841 |

N = Number of samples
Ho = Heterozygosity observed
He = Heterozygosity expected
HWE = Hardy-Weinberg Equilibrium ($\chi$2value< $\chi$2tab {0,05} means that the frequency of the genotypic population is under HWE condition)

**Table 3.** Allelic and Genotypic Frequencies of SCD1 Gene in Numerous Dairy Cow Breeds

| Breed | N | Allelic Frequencies | | Genotypic Frequencies | | | References |
|---|---|---|---|---|---|---|---|
| | | A | V | AA | AV | VV | |
| Isfahan Holstein | 408 | 0.58 | 0.48 | 0.26 | 0.65 | 0.09 | Nanaei et al. 2013 |
| Iranian Holstein | 394 | 0.76 | 0.24 | 0.60 | 0.32 | 0.08 | Mashhadi et al. 2012 |
| Canadian Jersey | 525 | 0.81 | 0.19 | 0.69 | 0.24 | 0.07 | Kgwatalala et al. 2009 |
| Italian Brown | 351 | 0.18 | 0.82 | 0.04 | 0.29 | 0.67 | Conte et al. 2010 |
| Italian Holstein | 297 | 0.57 | 0.43 | 0.27 | 0.60 | 0.13 | Mele et al. 2007 |
| Holstein | 143 | 0.71 | 0.29 | 0.50 | 0.42 | 0.08 | Clark et al. 2010 |
| Polish Holstein-Friesian | 100 | 0.77 | 0.23 | 0.58 | 0.36 | 0.06 | Kesek et al. 2017 |

The distribution of the SCD1|*Nco*I alleles is characterized by a higher frequency of the A allele compared to the V allele in most of HF dairy cow population studied (Table 2). Similar results with previous studies, Isfahan Holstein (0.58 vs 0.48) (Nanaei et al. 2013), Iranian Holstein (0.76 vs 0.24) (Mashhadi et al. 2012), Polish Holstein (0.77 vs 0.23) (Kesek et al. 2017). In contrastly, Italian Brown and Piedmontese breed from Italy have V allele higher than A allele (0.82 vs 0.18 (Conte et al. 2010) and 0.58 vs 0.42 (Moioli et al. 2007), respectively). Italian Brown cattle in Conte et al. (2010) study has positive of $F_{IS}$ value or inbreeding coefficient (0.05) comparing Italian Holstein (-0.23) that might caused genetic selection or the other cases. Based on $F_{ST}$ coefficient value for evaluating whether the effect of genetic selection or genetic drift, moderate $F_{ST}$ value was in between Italian brown and Italian Holstein (0.16) and high value was in between Italian brown cattle and Jersey (0.40). High $F_{ST}$ value indicated an elevated distance between Italian brown cattle and the other dairy breed. Macciotta et al (2008) reported that V allele higher than A allele also (0.566 and 0.434) with big population 538 Italian dairy cattle lactation.

Based on previous studies were known that SCD1|*Nco*I gene associated with fat milk content. Mele et al. (2007) studied milk fatty acid in Canadian Holstein and reported that AA genotype has higher in three fatty acid content (9.3% of *cis*-9 C14:1; 37.9% of *cis*-9 C18:1; and 11% of MUFA) than VV genotype. In CLA (*cis*-9, *trans*-11) concentration, AA genotype has higher than AV and VV genotypes (0.37; 0.36 and 0.33 g/100 g of total lipid, respectively). Herck (2009) investigated in Holstein that A allele was associated with higher proportion of C10:1, C12:1, C14:1, C18:0, and C18:1 trans 11 than V allele.

Different result has reported by Komisarek and Dorynek (Komisarek, J. and Dorynek 2009) which showed a positive effect of VV genotype on fat percentage. Futhermore, Macciotta et al. (2008) found that VV genotype has the greatest fat yield (1.22 kg/d) whereas AV (1.193 kg/d) and AA (1.186 kg/d) in Italian Holstein. Added by Conte et al. (2010) in Italian Brown cattle, VV allele has higher C14:1 cis 9 and DI 14 fatty acid (18.3% and 20.6%, respectively) than AV and AA genotypes. Higher proportion of C10:0, C12:0, C14:0, C16:1 and CLA was found in V allele of Holstein also (Herck, 2009).

Polymorphism of SCD1|*Nco*I gene in all of locations of HF dairy cows could be as early genetic information to improve of genetic quality through molecular breeding strategy. High of fat milk especially CLA and unsaturated fatty acid can be focus to get healthy milk.

## CONCLUSION

This study, demonstrated that there was a genetic polymorphism in samples collected from four locations of Friesian Holstein dairy cow population in Indonesia with dominance A allele. This finding may lead to further investigation on association of SCD1 gene polymorphism on milk fat yield in Friesian Holstein in Indonesia with higher sample of cows.

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# Productivity of Bali Cattle Fed Ration Supplemented by Molasses Containing Several Types of Defaunation Agents

Dinata AANBS, Pujiawati Y, Aurum S

*Balai Pengkajian Teknologi Pertanian Bali*
*Jalan By Pass Ngurah Rai, Pesanggaran, Denpasar Selatan-Bali 80222*
*E-mail : badunglahne@yahoo.co.id*

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**ABSTRAK**

Dinata AANBS, Pujiawati Y, Aurum S. 2019. Produktifitas sapi bali yang diberi pakan suplemen dengan molasses yang terdiri dari beberapa tipe agen defaunasi. JITV 24(2): 49-54. DOI: http://dx.doi.org/10.14334.jitv.v24i2.1958

Kombinasi molases dan agen defaunasi diindikasikan mampu meningkatkan daya cerna pakan. Penelitian ini bertujuan untuk mengkaji kombinasi molases dan agen defaunasi terhadap produktivitas Sapi Bali. Sapi Bali yang digunakan sebanyak 20 ekor dengan rata-rata bobot badan 307±52,46 kg dengan waktu penelitian selama enam bulan. Penelitian ini menggunakan rancangan acak lengkap dengan 4 perlakuan dan 5 ulangan. Perlakuan yang diberikan yaitu P1 : rumput gajah + polard 1,5 kg/ekor/hari, P2 : rumput gajah + polard 1,5 kg/ekor/hari + *Hibiscus tiliaceus moladef* 10 cc/ekor/hari, P3 : rumput gajah + polard 1,5 kg/ekor/hari + *Hibiscus rosasinensis moladef* 10 cc/ekor/hari, P4 : rumput gajah + polard 1,5 kg/ekor/hari + Aloe vera moladef 10 cc/ekor/hari. Parameter yang diamati adalah rata-rata pertambahan bobot badan harian, konsumsi pakan, feed conversion ratio (FCR) dan kecernaan pakan. Hasil penelitian ini menunjukan rata-rata pertambahan bobot badan harian paling baik terdapat pada perlakuan P2 yaitu 543,13 g/ekor/hari. Perlakuan P2 juga menunjukan kecernaan bahan kering dan serat kasar yang tinggi yaitu 81,36% dan 73,58%. Disimpulkan bahwa Sapi Bali yang diberikan rumput gajah + polard 1,5 kg/ekor/hari + *Hibiscus tiliaceus moladef* 10 cc/ekor/hari menghasilkan pertambahan bobot badan harian dan kecernaan serat kasar tertinggi.

**Kata Kunci**: Kecernaan, Moladef, Produktivitas, Sapi Bali

**ABSTRACT**

Dinata AANBS, Pujiawati Y, Aurum S. 2019. Productivity of Bali Cattle fed ration supplemented by molasses containing several types of defaunation Agents. JITV 24(2): 49-54. DOI: http://dx.doi.org/10.14334.jitv.v24i2.1958

Defaunation agents and molasses combination indicate able to improve digestibility of feed. This study was conducted to assest productivity of Bali Cattle fed ration supplemented with molasses solution containing several types of defaunation agents (moladef). Twenty Bali Cattle with average body weight of 307.56±52.46 kg were used in this research for six months. This study was arranged in a completely randomized design with four treatments and five replications. The treatments were P1 : Napier grass+pollard 1. 5 kg/head/day, P2 : napier grass + pollard 1. 5 kg/head/day + Hibiscus tiliaceus moladef 10 cc/head/day, P3 : napier grass + pollard 1. 5 kg/head/day + Hibiscus rosasinensis moladef, P4 : napier grass + pollard 1. 5 kg/head/day + Aloe vera moladef 10 cc/head/day . The parameters observed were average daily gain (ADG), feed intake, feed conversion ratio (FCR) and feed digestibility. This study suggest that the best average daily gain (ADG) was found in treatment P2 is 543,13 g/head/day. Treatment P2 also showed highest dry matter digestibility and crude fiber digestibility was 81,36% and 73,85%. It is concluded that Bali Cattle fed on napier grass + pollard 1. 5 kg/head/day + Hibiscus tiliaceus moladef 10 cc/head/day resulted in the highest ADG and CF digestibility.

**Key Words**: Bali Cattle, Digestibility, Moladef, Productivity

## INTRODUCTION

Bali Cattle according to their name are from Bali province, which spread from the 1990s (Talib 2002). Bali Cattle have a several advantages such as having high adaptability and good reproductive performance (Talib 2002). The average body weight gain of Bali Cattle according to Panjaitan et al. (2014) is ranging from 0.23-0.61 kg/day, whereas according to Rauf et al. (2015) Bali cattle which only consumed forage in grazing systems was only able to produce average daily gain 0.15 kg/day.

The use of natural pasture and crop residues as the main feed is not sufficient to meet nutrient requirements, especially in the growth phase of Bali Cattle. This is indicated by poor feed digestibility so that nutrient intake for growth is not met sufficient. The crude fiber content in forage is more than 20 %, so fiber digestibility plays an important role in supporting the ruminant's performance. Studies to improve forage

digestibility have been widely carried out such as processing physically, chemically and biologically so that forage is easily digested and absorbed by animal body (Wanapat et al. 1996). In addition there are alternative efforts by using several tropical local plants that are known to be able to improve the performance of ruminant's digestive systems (Santra & Karim 2003).

Ruminants are able to degrade and use fibrous feed as a source of energy and nutrients because of the presence of complex anaerobic microbiota in the rumen, composed mainly of bacteria, fungi, and ciliate protozoa (Durand & Ossa 2014). Each rumen microbes has a specific function on feed digestibility. Arora (1995) stated that feed digestibility increase if the rumen microbial population increase, especially the cellulose and hemicellulose digestive bacteria.

Saponin is a natural phytochemical compound that has been widely studied to improve rumen metabolism so that it has a positive impact on increasing the efficiency of ruminant production. The three local plant such as namely *Hibiscus tiliaceus, Hibiscus rosasinensis* and *Aloe vera* are known to have natural phytochemical compounds in the form saponins (Istiqomah et al. 2011; Widiawati et al. 2017; Kusumastuti & Yuniar 2016)**.** A study of utilization *Hibiscus tiliaceus* leaf at 10% level can decrease protozoa population and gas production, and there was no effect on NH3 and VFA concentration also pH value (Istiqomah et al. 2011). Study by McMurphy et al. (2014) showed that use of saponin from extract *Yucca schidigera* as Micro-Aid® in protein supplements increase rumen dry matter and a NDF digestibility in steers fed low quality prairie hay.

Saponin content in *Hibiscus tiliaceus, Hibiscus rosasinensis* and *Aloe vera* is indicated to reduce ration palatibility. According to Santoso & Sartini (2001) the content of saponin in *Sauropus androgynus* leaf can reduce ration palatability because it has a bitter taste and is smooth. To prevent this problem, the combination of the three plants with molasses is an attempt to reduce the chances of decreasing ration palatability. Molasses is suitable for inclusion in the diets of all ruminant livestock and effective to increase the palatability of feeds whilst contributing for increased levels of energy and protein (Senthilkumar et al. 2016). Molasses is a available energy source for microbes and also serves as to protect proteins protection from being entirely degraded in the rumen (Supriyati, Haryanto 2011; Kardaya et al. 2009; Mathius 2009).

Based on briefly literature mentioned above, it is necessary to conduct a study on the addition of molasses solutions containing defaunation agent to increase productivity of Bali cattle. The research was conducted to determine productivity and nutrient digestibility of Bali cattle fed ration supplemented with molasses solution containing several types of defaunation agents (Moladef).

## MATERIALS AND METHODS

In vivo research was conducted at "Rare Angon" farmer group in Gel-gel village, Klungkung Sub-district, Klungkung district. Research was conducted six months with preliminary study for one week. For experiment, 20 Bali Cattle was used with mean body weight 307.56 ± 52.46 kg in individual cages. The cage was concrete ground equipped with drinking and feeding facility. Feeding was offered twice a day at 08.00 am and 04.00 pm. Experimental design applied was completely randomized design consisted of 4 feeding treatments with 5 replicates. Feeding treatments were : P1 : napier grass + pollard 1.5 kg/head/day, P2 : napier grass + pollard 1.5 kg/head/day + *Hibiscus tiliaceus* moladef 10 cc/head/day, P3 : napier grass + pollard 1.5 kg/head/day + *Hibiscus rosasinensis* moladef 10 cc/head/day, P4 : napier grass + pollard 1.5 kg/head/day + *Aloe vera* moladef 10 cc/head/day.

This experiment use 4 L of water available for cattle and will be refill once consumed. The diet is given separately between napier grass and pollard.

Fresh napier grass was given as much as 10% of body weight. Napier grass was harvested at 40 days of age, and chopped into 5 – 10 cm length. Pollard was fed in the morning prior to Napier grass. Moladef is a solution consisting of molasses with a defaunation agent. Moladef was given by drinking water at the same time as forage feeding. Moladef is given per day as much as 10 cc/head/day diluted in 4 liters of drinking water, drinking water consumed entirely.

Moladef preparation started by dissolving molasses solution with clean water. 700 ml of water was added to 300 ml molasses followed by homogenization. Defaunation agent was mixed in the solution form by initial preparation of 50 g dry of materials (*Hibiscus tiliaceus, Hibiscus rosa-sinensis,* or *Aloe vera)* grinded and milled with mechanical grinder followed by adding 1 L of water then filtered. The filtrate was added to molasses solution at concentration 20% of total volume and then mixed well.

Data recorded were (1) average daily weight, (2) feed intake (3) Feed Conversion Ratio (FCR) and (4) feed digestibility. Weight increase determined by measuring the difference between monthly body weight. Average daily gain quantified by deducting the final body weight with initial body weight devided by the time of research.

**Table 1.** Nutrient composition of experimental diets in total mix ration

| Nutrients | Experimental diets | | | |
|---|---|---|---|---|
| | P1 | P2 | P3 | P4 |
| Dry Matters (DM) | 28.00 | 28.27 | 27.74 | 28.21 |
| Organic Matters (OM) | 88.56 | 89.24 | 87.49 | 87.48 |
| Ash | 11.44 | 10.76 | 12.51 | 12.52 |
| Crude Protein (CP) | 13.38 | 13.78 | 13.53 | 13.40 |
| Fat | 2.12 | 2.79 | 2.76 | 2.75 |
| Crude Fiber (CF) | 20.35 | 17.59 | 18.62 | 18.46 |
| Energy (kkal/kg) | 2538 | 2670 | 2604 | 2601 |
| Nitrogen Free Extract (NFE) | 17.19 | 16.70 | 15.96 | 15.96 |
| Total Digestible Nutrient (TDN) | 54.69 | 54.35 | 54.32 | 54.00 |

P1 = Napier grass + pollard 1.5 kg/head/day;
P2 = Napier grass + pollard 1.5 kg/head/day + *Hibiscus tiliaceus* moladef 10 cc/head/day;
P3 = Napier grass + pollard 1.5 kg/head/day + *Hibiscus rosa-sinensis* moladef 10cc/head/day;
P4 = Napier grass + pollard 1.5 kg/head/day + *Aloe vera* moladef 10 cc/head/day.

Feed intake measured based on dry matters intake (kg/head/day) calculated by finding the difference of feed given with daily residual feed (Parakkasi, 1999). FCR quantification conducted by dividing number of daily ration with weight increase per cow per day during the research. Digestibility is calculated by the formula :

$$\frac{\text{Nutrien intake (kg/DM) - Nutrien in fecal (kg/DM)}}{\text{Nutrien intake (kg/DM)}} \times 100\%$$

The samples of Napier grass and polard offered and refusals samples were collected during the last 7 days of each period by using total collection method. Fecal samples were collected during the last 7 days of each period by using total collection method as animal were moved to the metabolic crates. Feed sample were withdrawn 10% of the given, and fecal withdrawn 5% from the total production. Feed and fecal samples were sun dried followed by composite process and then taken out 200 g from each treatment for laboratory analysis i.e. DM and nutrition content. DM of feed and fecal determined with AOAC method (AOAC 2005). Analysis of crude protein using the Kjedahl method was through the process of destruction, distillation, titration and calculation, while for crude fat the method used included extraction of soxhlet with solvent fat petroleum ether. Analysis of crude fiber using acid solvents and dilute bases was through boiling each of 30 minutes. Energy measurement using the Bomb Calorimeter by measuring changes in temperature due to combustion. NFE value obtained from the calculation of 100% - (Water + Ash + Crude Protein + Crude fat + Crude Fiber %). Total digestible nutrient value was obtained from calculations using formula by Wardeh (1981).

Experemental data obtained were analyzed with one way ANOVA or completely randomized design with four treatment and five replications. Data were analyzed using the model $Yij = \mu + \tau i + eij$. Where Yij is the variable under consideration, $\mu$ the overall mean, Ti the ith treatment and eij is the residual error. Differences between treatment means were determined by Duncan's Multiple Range Test, and differences among means with $p < 0.05$ were represented as statistically significant different.

## RESULTS AND DISCUSSION

### Productive Performance and Nutrient Intake

Application of Moladef (molasses containing defaunation agent) has positive effect to average daily gain (ADG) of Bali Cattle. The average daily gain (ADG) increase of Bali Cattle under P2 treatment was 543.13 g/head/day or 30. 53% and 17.28% higher than P1 and P4 (Table 2). Average daily gain in this study was 29% higher than ADG for Bali Cattle as reported by Panjaitan et al. (2014) being 230-610 g/head/day which was fed with *Leucaena leucocephala*. The FCR value is determined based on the amount of ration consumed divided by daily body weight gain. The FCR value was not significantly different between the treatments. The FCR value were not significantly different indicates that the ration used has not been efficient in changing feed consumption into daily body

weight gain, if the highest daily body weight gain is observed in P2, it is supported by a low FCR value.

Feed intake in Table 2 shows no significant different (P>0.05) among treaatments. Feed intake (as fed) ranging from 34,06-34,99 kg/head/day or 11% BB and dry matter intake ranging from 9,59-9,71 kg/head/day. Feed intake was not significantly different between treatments but having different effect on production performance indicates the digestibility of feed form each treatments plays an important role in production performance as indicated by daily weight gain.

These result also showed that supplementation of *Hibiscus tiliaceus* as moladef in P2 had a positive impact on daily body weight gain compared to other type of plants as saponin source. According to Bata et al. (2016) stated that the addition of 0.48% waru leaf meal (*Hibiscus tiliaceus*) on Sumba Ongol Cattle diet did not increase dry matter intake, organic matter digestibility but has a trend to increase daily body weight gain and feed efficiency.

In this study average daily gain in P2 was higher than other diet, this indicated that saponin in *Hibiscus tiliaceus* plant were thought to be more effective than other treatments. Istiqomah et al. (2011) stated that the use of waru leaf (*Hibiscus tiliaceus*) as much as 10% in ration was the most optimal to improve rumen fermentation for propionate synthesis, reduce protozoa population, reduced gas production and did not negatively affect to $NH_3$ concentration, VFA concentration and pH value. This is also in line with the in vitro study, that use of *Hibiscus tiliaceus* as moladef has increased propionate synthesis, $NH_3$ concentration, dry matter digestibility and organic matter digestibility (Dinata & Pujiawati 2018).

**Table 2**. Bali cattle average daily gain suplemented by moladef

| Parameter[1)] | Treatments | | | |
|---|---|---|---|---|
| | P1 | P2 | P3 | P4 |
| Initial body weight (kg) | 307.90 ± 42.03 | 309.50 ± 45.59 | 306.70 ± 55.11 | 306.20 ± 59.79 |
| Final body weight (kg) | 382.30 ± 31.13 | 406.70 ± 40.00 | 389.70 ± 44.41 | 383.50 ± 58.53 |
| Average daily gain (g/head/day)* | 416.18 $^{a}$ ± 26.58 | 543.13 $^{b}$ ± 24.55 | 463.25 $^{ab}$ ± 29.24 | 432.31 $^{a}$ ± 27.59 |
| Feed intake (kg/head/day) | | | | |
| Wet weight | 34.24 ± 2.28 | 34.36 ± 5.60 | 34.99 ± 6.10 | 34.06 ± 4.43 |
| Dry mater | 9.59 ± 0.64 | 9.71 ± 1.58 | 9.71 ± 1.69 | 9.61 ± 1.25 |
| FCR | 24.30 ± 6.53 | 18.40 ± 4.65 | 21.11± 4.29 | 23.68 ± 5.95 |

[1)] Values with different superscripts on the same rows show a significant difference (P<0.05)

**Table 3**. Feed intake of Bali cattle suplemented by moladef

| Parameter (kg/head/day)[1)] | Treatments | | | |
|---|---|---|---|---|
| | P1 | P2 | P3 | P4 |
| DM | 9.59 ± 0.64 | 9.71 ± 1.58 | 9.71 ± 1.69 | 9.61 ± 1.25 |
| OM | 7.47 ± 0.50 | 7.63 ± 1.24 | 7.46 ± 1.30 | 7.37 ± 0.96 |
| CP | 1.28 ± 0.09 | 1.34 ± 0.23 | 1.31 ± 0.23 | 1.29 ± 0.17 |
| Fat | 0.20$^{a}$ ± 0.01 | 0.27 $^{b}$ ± 0.04 | 0.27 $^{b}$ ± 0.05 | 0.26 $^{b}$ ± 0.03 |
| CF | 1.95 ± 0.13 | 1.71 ± 0.28 | 1.81 ± 0.32 | 1.77 ± 0.23 |
| Energy (kkal/kg) | 86.90 ± 5.78 | 91.73 ± 14.96 | 91.11 ± 15.89 | 88.59 ± 11.53 |
| NFE[2)] | 1.65 ± 0.11 | 1.62 ± 0.26 | 1.55 ± 0.27 | 1.53 ± 0.20 |
| TDN | 5.71± 0.38 | 5.64 ± 0.92 | 5.64 ± 0.98 | 5.55 ± 0.72 |

1) Values with different superscript in the same rows show a significant difference (P<0.05)
2) Values with different letters on the same rows show a highly significant difference

Dry matter (DM) intake in cattle treated with moladef was not significantly different (P>0.05) among treatment (Table 2). Moladef supplementation in drinking water does not affect dry matter intake indicating that saponin content in moladef does not reduce ration palatability. Suharti et al. (2009) stated that the saponin in the diet can decrease palatability because it has a bitter taste. This is indicated that molasses content in moladef can overcome the bitter taste of saponin. Senthilkumar et al. (2016) stated that the provision of molasses in diet increased the palatability of feed, thus increasing feed intake.

Suplementation moladef did not affect to nutrient consumption (Table 3) except crude fat consumption (P<0.05). This is because the crude fat contained in P1 treatment lower than other treatments. Crude fat intake in this study did not affect to average daily gain (ADG) Bali cattle (Table 2). Suplementation moladef did not affect to nutrient consumption because nutrient in experiment diets designed to be iso-energy and iso-protein. TDN and protein consumption is relatively similar in all treatments.

TDN consumption (Table 3) is still within the standard range of Kearl (1982) which states that TDN consumption for cattle with body weight 300 kg and daily body weight gain 0.5 kg is 3.8 kg, while for crude protein consumption also still within the standard range crude protein consumption is 604 gram or 0.6 kg. Crude fiber consumption in this study ranged from 1.71-1.95 kg/head/day (P>0.05). This is because the crude fiber content in ration is not too different between diets, but the crude fiber digestibility is more optimal with moladef treatment (Table 4).

**Nutrient Digestibility**

The addition of moladef was not significant effect on nutrient digestibility such as dry matter, organic matter, crude protein, crude fat and TDN (P>0.05; Table 4). The addition of moladef has an influence on the NFE digestibility and crude fiber digestibility (P<0.05; Table 4). This is indicated that 10 cc/head/day moladef in diets did not have negative effect on nutrient digestibility. In vitro studies, dry matter digestibility for moladef treatment was higher 18.33-26.55 % compared without moladef treatment, whereas for organic matter digestibility was higher at moladef treatment ie 22.76 – 33.61% compared to control (Dinata & Pujiawati 2018). These results are inversely proportional to digestibility of dry matter and organic matter in the in-vivo study, for which further studies are needed regarding the effectiveness of moladef in the in-vivo study.

## CONCLUSION

The Suplementation of *Hibiscus tiliaceus* moladef 10 cc/head/day particularly in Bali cattle fed Napier grass + pollard 1.5 kg/head/day has the highest productivity and can increase crude fiber digestibility.

**Table 4**. Nutrient digestibility of Bali cattle Supplemented by Moladef

| Digestibility (%) [1)] | Treatments | | | |
|---|---|---|---|---|
| | P1 | P2 | P3 | P4 |
| DM | 79.07 ± 3.95 | 81.36 ± 2.91 | 79.93 ± 3.97 | 78.31 ± 3.06 |
| OM | 79.96 ± 3.78 | 82.39 ± 2.75 | 81.04 ± 3.75 | 79.15 ± 2.95 |
| CP | 87.07 ± 2.44 | 87.73 ± 1.92 | 87.67 ± 2.44 | 86.87 ± 1.86 |
| Fat | 84.23 ± 2.98 | 86.26 ± 2.15 | 90.75 ± 1.83 | 88.23 ± 1.66 |
| CF | 52.52 [a] ± 8.96 | 73.58 [b] ± 4.13 | 63.58 [ab] ± 7.20 | 61.11 [ab] ± 5.49 |
| Energy (kkal/kg) | 92.00 ± 1.51 | 93.35 ± 1.04 | 92.92 ± 1.40 | 92.20 ± 1.10 |
| NFE [2)] | 78.52 [b] ± 4.05 | 59.50 [a] ± 6.33 | 65.83 [a] ± 6.75 | 59.20 [a] ± 5.76 |
| TDN | 89.62 ± 1.96 | 88.97 ± 1.72 | 87.99 ± 2.37 | 86.19 ± 1.95 |

[1)] Values with different letters on the same line show a significant difference
[2)] Values with different letters on the same line show a highly significant difference

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# Enhance *in-vitro* rumen fermentation of *Panicum maximum* with biological supplements

Chathurika APS, Sujani S, Manawadu A and Seresinhe T

*Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka*
*E-mail:* sujani@ansci.ruh.ac.lk

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**ABSTRAK**

Chathurika APS, Sujani S, Manawadu A and Seresinhe T. 2019. Upaya meningkatkan fermentasi rumen *Panicum maximum* dengan suplemen biologis secara in vitro. JITV 24(2): 82-86. DOI.: http://dx.doi.org/10.14334.jitv.v24i2.1963

Akhir-akhir ini, penggunaan pakan suplemen biologis meningkat dibandingkan penggunaan pakan suplemen kimia. Tujuan objek penelitian ini adalah untuk mengevaluasi pengaruh pemberian suplemen rumput *Panicum maximum*. dengan dua spesies tanaman: *Artocarpus heterophyllus* (Jack leaves; AH) dan *Tridax procumbens* (TP) yang mengandung tanaman sekunder yang masing-masing dapat memetabolisme tannin dan saponin dan enzim yang memproduksi Dyadic Cellulase (CE) dan Ragi (YE). Dilakukan uji terhadap dua level perlakuan pada masing-masing suplemen. Untuk suplemen berbasis 20 (AHT1, TPT1) dan 30% (AHT2 dan TPT2) tanaman disubtitusi dengan subtrat basa. Enzim yang digunakan adalah sebanyak 10 µl (CET1) dan 20 µl (CET2) serta ragi sebanyak 4 mg (YET1) dan 6 mg (YET2). Penelitian ini menggunakan Rancangan Acak Kelompok Lengkap (RAK) dengan lama inkubasi fermentasi *in-vitro* rumen selama 72 jam. Semua perlakuan secara nyata meningkatkan produksi gas *in-vitro* (PGIV) dibandingkan dengan kontrol. Perlakuan AH dan CE secara nyata meningkatkan degradibilitas bahan kering rumen in vitro (DBHIVR). Produksi nitrogen ammonia ($NH_3$-N) tidak dipengaruhi oleh suplemen. Dapat disimpulkan bahwa perlakuan dalam penelitian ini memperkaya fermentasi rumen dalam hal PGIV dan DBHIVR dan mengurangi jumlah protozoa.

**Kata Kunci**: Artocarpus heterophyllus, Tridax procumbens, Selulase, Ragi, Protozoa, Fermentasi Rumen

**ABSTRACT**

Chathurika APS, Sujani S, Manawadu A and Seresinhe T. 2019. Enhance in-vitro rumen fermentation of *Panicum maximum* with biological supplements. JITV 24(2): 82-86. DOI.: http://dx.doi.org/10.14334.jitv.v24i2.1963

Recently the utilization of biological feed additives over chemical feed additives in animal feeds have increased. The objective of the present study was to evaluate the effect of supplementing wild guinea grass (*Panicum maximum*) with two plant species, *artocarpus heterophyllus* (jack leaves; ah) and *tridax procumbens* (TP) containing plant secondary metabolites tannin and saponin, respectively and the enzyme product dyadic cellulase (CE) and yeast (YE). For each suplement two levels of treatments were tested. In plant-based suplements 20 (AHT1, Tpt1) and 30% (AHT2 and Tpt2) substituted the base substrate. The enzyme was applied as 10 µl (CET1) and 20 µl (CET2) and yeast as 4 mg (YET1) and 6 mg (YET2). the experimental design was a randomized complete block design (RCBD) and the period of in vitro rumen fermentation incubation was 72 hrs. All treatments significantly ($P < 0.05$) enhanced the in vitro gas production (IVGP) compared with the control. Treatments of ah and ce significantly ($P < 0.05$) improved the in vitro rumen dry matter degradability (IVRDMD). All treatments significantly ($P<0.05$) suppressed the ruminal protozoa population as compared to the control. Ammonia nitrogen (NH3-N) production was not significantly ($P>0.05$) influenced with supplements. in conclusion, treatments enhanced the rumen fermentation in means of enhanced IVGP, IVRDMD and reduced protozoa numbers.

**Key Words**: Artocarpus heterophyllus, Tridax procumbens, Cellulase, Yeast, Protozoa, Rumen Fermentation

## INTRODUCTION

The gap between the nutrient requirement and the low nutrient availability combined with the poor digestibility of the commonly available feedstuffs is the major constraint against better performance and optimum production of ruminant animal in the tropics. One of the sustainable and widely distributed fodder species in the tropics is guinea grass which is characterized with several poor characteristics such as lower digestibility of the fiber fraction, drastic reduction of crude protein content with the maturity and low crude, suggests a need for supplementation. Enhancing the digestibility of fiber component of ruminant feedstuffs has been a huge research interest among ruminant nutritionists; thus various chemical, physical and microbiological methods have been introduced and some are being practically implemented. As evidenced by several studies, exogenous fibrolytic enzyme cellulase has several advantages in improving rumen fermentation parameters (Sujani et al. 2015). Despite the positive results gained with cellulase enzyme the relatively high cost and the less awareness have hindered the usage it under small-scale livestock farmers in tropics. This limitation has created an opportunity to research on less expensive and widely available biological supplements. In this backdrop utilization of plants and

shrubs containing plant secondary metabolites and direct fed microbial, two biological approaches were tested. Supplementations of guinea grass with tree foliage containing plant secondary metabolites such as tannin and saponin were reported (Babayemi 2007) to improve the performance of ruminants. Yeast culture supplementation has been reported to enhance microbial growth and decrease N loss by incorporating more digestible carbohydrates into microbial mass (Sniffen et al. 2004). Therefore, the objective of the present study was to determine the effect of above-mentioned supplements on *in vitro* rumen fermentation parameters, *in vitro* gas production (IVGP), *in vitro* rumen dry matter degradability (IVRDMD), rumen protozoa population and ammonia nitrogen ($NH_3$-N) production of wild guinea grass.

## MATERIALS AND METHODS

### Substrate and supplement preparation

Guinea grass (*Panicum maximum*), *Artocarpus heterophyllus* and *Tridax procumbens* were collected, dried (55°C for 48 hours) and ground to pass 1 mm screen. Other supplements were yeast (*Saccharomyces cerevisiae*) and Cellulase (CE) (E.C. 3.2.1.4,) produced by the fermentation of non-genetically modified organisms *Trichoderma longibrachiatum*. Proximate analysis of dry matter (DM) and crude protein (CP) were done according to the AOAC (1990).

### Treatment Allocation

There are eight treatment combinations that will be compared with controls as presented in Table 1.

### Experimental design

The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replicates for each treatment and experiments were repeated twice.

### Statistical Analysis

Analysis of variance (ANOVA) was performed on IVGP, IVRDMD, $NH_3$-N, NDFD and protozoa count with SPSS 20.0 statistics package and the statistical significance of the differences between means was tested using the Least Significant Difference (LSD). Descriptive analysis was done using Microsoft Excel 2010 version.

### *In vitro* gas production technique

*In vitro* fermentation procedure and preparation of buffer and mineral solutions were done according to the procedures demonstrated by Menke & Steingas (1988). Samples (base substrate and supplement) were accurately weighed into glass bottles (120 ml).

For the *in vitro* incubation procedure, the medium of 1l volume was prepared with 2.5 g of tryptone (Sigma-Aldrich, Co., 3050 Spruce Street, St. Louis, MO, USA) dissolved in 500 ml distilled water, 0.125 ml of micro mineral solution, 250 ml of buffer solution and 1.25 ml of 0.1% (w/v) resazurin (Fluka AG, CH-9470 Buchs, Switzerland) solution. The medium was mixed in a container which kept in a water bath (39°C) while bubbling $CO_2$ through the solution for 45 minutes. L-cysteine hydrochloride (0.313 g) (Sigma-Aldrich, Co., 3050 Spruce Street, St. Louis, MO, USA) and sodium sulfide (0.313 g) (Park Scientific Limited, Northampton, UK) were directly added to the medium and further bubbled with $CO_2$ for 15 min. At this point, rumen fluid was collected from two donor heifers at the faculty farm (Faculty of Agriculture, University of Ruhuna, Kaburupitiya, Sri Lanka) through an esophageal suction method. Collected rumen fluid was transferred to a pre-warmed flask and strained through four layers of cheese cloth. All the laboratory handlings of rumen fluid were carried out under a continuous flow of $CO_2$ and 39°C of temperature. Prepared rumen fluid was added to the medium in a ratio of 1:4 (rumen fluid: medium) and flushing of $CO_2$ was continued until the solution turned to grey or clear, after which 42 ml of medium were pipetted into each incubation bottle, containing the pre-incubated substrate, and the bottles were immediately crimp sealed with a rubber stopper and placed in the water bath with shaker at 39ºC.

### Measurements and data collection

*In vitro* gas production was recorded at 3, 6, 9, 12, 24, 48 and 72 h within the incubation period. After 72 h, bottles were removed from the shaker and placed on ice to terminate the reaction. Remaining solid portions were separately prepared to determine IVRDMD and NDFD while the aliquots of the filtrates were stored at $20^0$C until analyzed for $NH_3$-N and protozoan count (by mixing 1 ml of the filtrate with a 1 ml of 40 % (w/v) formaldehyde).

### Chemical Analysis

At the end of incubation, solid portions were separately analyzed to determine IVRDMD with oven

**Tabel 1.** Combination Of Treatment

| Treatment | Level of treatment | Amount of treatment (mg) | Amount of base substrate (*Panicum maximum*) (mg) |
|---|---|---|---|
| AHT1 | *Artocarpus heterophyllus* (20%) | 100 mg | 400 mg |
| AHT2 | *Artocarpus heterophyllus* (30%) | 150 mg | 350 mg |
| TPT1 | *Tridax procumbens* (20%) | 100 mg | 400 mg |
| TPT2 | *Tridax procumbens* (30%) | 150 mg | 350 mg |
| CET1 | Cellulase (CE) | 10 µl | 500 mg |
| CET2 | Cellulase (CE) | 20 µl | 500 mg |
| YET1C | Yeast (YE) | 4 mg | 500 mg |
| YET2C | Yeast (YE) | 6 mg | 500 mg |
| Control | _ | _ | 500 mg |

dry method ($55^0$C, 48 hours) and NDFD was estimated following methods of Van Soest (1963). Liquid portion was analyzed for $NH_3$-N (Kjeltec System 1002, Tecator AB, Hoganas, Sweden) (AOAC 1990) and protozoa were counted with Burker type hemocytometre (0.1 and 0.02 mm depth, respectively; Blau Brandw, Wertheim, Germany). Triplicate preparations of each sample were counted

## RESULTS AND DISCUSSION

### Result

The chemical composition of substrate Guinea Grass and plants which supplied supplements are presented in Table 2. Data on IVGP, IVRDMD, NDFD, $NH_3$-N and protozoa count are presented in Table 3. IVGP supplemented with AHT1, AHT2, TPT1, YET1, YET2 and CET2 was significantly higher when compared with control.

Only CE and AHT2 could significantly increase the IVRDMD where upper levels of AH and TP significantly reduce the NDFD. No significant difference was observed in $NH_3$-N production with any treatment. With all treatments protozoa count was significantly reduced.

### Discusion

Gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs as it helps to better quantify nutrients utilization hence a good indicator of digestibility, fermentability, and microbial protein production (Getachew et al. 1998). Referring to the Figure 1, it could be assumed that accelerated initial IVGP upto 3 hours of incubation could be due to the stimulation of initial phase degradation of substrate (Giraldo et al. 2007). When consider all supplements it was observed that there was a drop in gas production with AH and TP supplements and it suggests a negative effect of tannin and saponin on *in vitro* gas production whereas CE showed a positive effect on *in vitro* gas production and it would be the most likely mode of action of enzyme. Finding on the negative relationship between phenolics and dry matter degradation is in line with the present results where a high level of saponin (TPT2) significantly reduced the IVRDMD. The effectiveness of yeast addition on *in vitro* fermentation parameters showed inconsistencies in previous studies. Mutsvangwa et al. (1992) reported that *in vitro* GP of a barley diet for beef cattle supplemented with yeast culture (Yea-Sacc1026) was lower than that in the control, while Tang et al. (2008) found that supplementation of yeast culture (Original XP; Diamond V Mills Inc., Cedar Rapids, IA, USA) increased the cumulative GP and present results are consistent with the latter finding. Differences in the yeast species derived from may be the main reason for discrepancies in results whereas fermentation substrate and experimental conditions could also contribute. Significantly enhanced IVGP upon the supplementation of CE was reported by Eun & Beauchemin (2007) and in contrary Giraldo et al. (2007) found that there was no significant effect of enzyme supplementation on IVGP of a fibrous diet. As suggested by a previous study of Colombatto & Beauchemin (2003), enzymes could enhance IVRDMD by removing structural barriers and facilitating microbial colonization resulting the increased rate of degradation, which is consistent with current research results.

Dietary protein is fermented in the rumen to simpler N compounds and reincorporated; primarily as $NH_3$-N which acts as an indicator of microbial nitrogen synthesis. As $NH_3$-N is the primary N source of most ruminal organisms increased $NH_3$-N could be resulted from improved microbial activities. Fadel Elseed et al. (2007) reported yeast (*S. cerevisiae*) supplementation resulted in a numerical increase in ammonia-N concentration in rumen fluid of Nubian goat's kids through any change could not be observed in the present study. Wang et al. (2001) found that no effect of fibrolytic enzyme supplementation on ruminal $NH_3$-N production under in vitro conditions and present results agree with the findings.

**Table 2.** Chemical Composition of Substrate Guinea Grass (*Panicum Maximum*) And Supplements

| Substrate | DM (g/ kg) | CP (g/ kg DM) | NDF (g/ kg DM) |
|---|---|---|---|
| *Panicum maximum* | 283 ± 3.37 | 214.0 ± 4.30 | 619 ± 6.50 |
| *Artocarpus heterophyllus* | 202 ± 2.25 | 221.1 ± 0.31 | 342 ± 5.61 |
| *Tridax procumbens* | 268 ± 10.21 | 345.7 ± 0.05 | 495 ± 7.21 |

**Table 3.** Cumulative *in vitro* gas production (IVGP), *in vitro* rumen dry matter disappearance (IVRDMD), neutral detergent fiber disappearance (NDFD), ammonia nitrogen ($NH_3$-N) production and protozoan count and of guinea grass (*Panicum maximum*) in response to the treatments.

| Treatment | IVGP (ml/ 500 mg DM/72 hr) | IVRDMD % | NDFD % | $NH_3$-N mg/100ml | Protozoa Count/1ml |
|---|---|---|---|---|---|
| AHT1 | 75±0.38$^{b}$ | 69.43±1.07$^{a}$ | 52.14±1.87$^{b}$ | 44.71±0.34$^{a}$ | 5556±103$^{b}$ |
| AHT2 | 75±0.45$^{b}$ | 71.97±1.42$^{d}$ | 46.25±1.53$^{c}$ | 42.16±2.89$^{a}$ | 7778±111$^{b}$ |
| TPT1 | 76.5±0.87$^{b}$ | 69.43± 0.86$^{a}$ | 50.82±1.80$^{b}$ | 45.56±1.02$^{a}$ | 6667±222$^{b}$ |
| TPT2 | 72±2.24$^{a}$ | 63.10±2.91$^{e}$ | 52.14±1.45$^{b}$ | 45.90±0.85$^{a}$ | 3333±98$^{c}$ |
| YET1 | 75.5±0.29$^{b}$ | 68.97±0.60$^{a}$ | 52.06±1.78$^{b}$ | 44.37±2.21$^{a}$ | 4444±210$^{c}$ |
| YET2 | 78.5±0.36$^{c}$ | 69.33±0.37$^{a}$ | 54.08±2.10$^{a}$ | 46.41±0.51$^{a}$ | 3333±86$^{c}$ |
| CET1 | 73±1.25$^{a}$ | 73.47±2.42$^{b}$ | 56.20±3.97$^{a}$ | 47.09±5.95$^{a}$ | 6667±133$^{b}$ |
| CET2 | 80.5±1.83$^{c}$ | 71.93±2.89$^{c}$ | 54.66±3.57$^{a}$ | 45.39±1.87$^{a}$ | 6667±104$^{b}$ |
| Control | 73±0.79$^{a}$ | 67.80±0.94$^{a}$ | 54.51±1.95$^{a}$ | 45.73±2.72$^{a}$ | 12222±276$^{a}$ |

Values are means of three replicates ± SE. The significance of means was considered at $p < 0.05$.
AHT1 = 20% AH TPT1 = 20% TP YET1 = 4 mg CET1 = 10 µl
AHT2 = 30% AH TPT2 = 30% TP YET2 = 6 mg CET2 = 20 µl

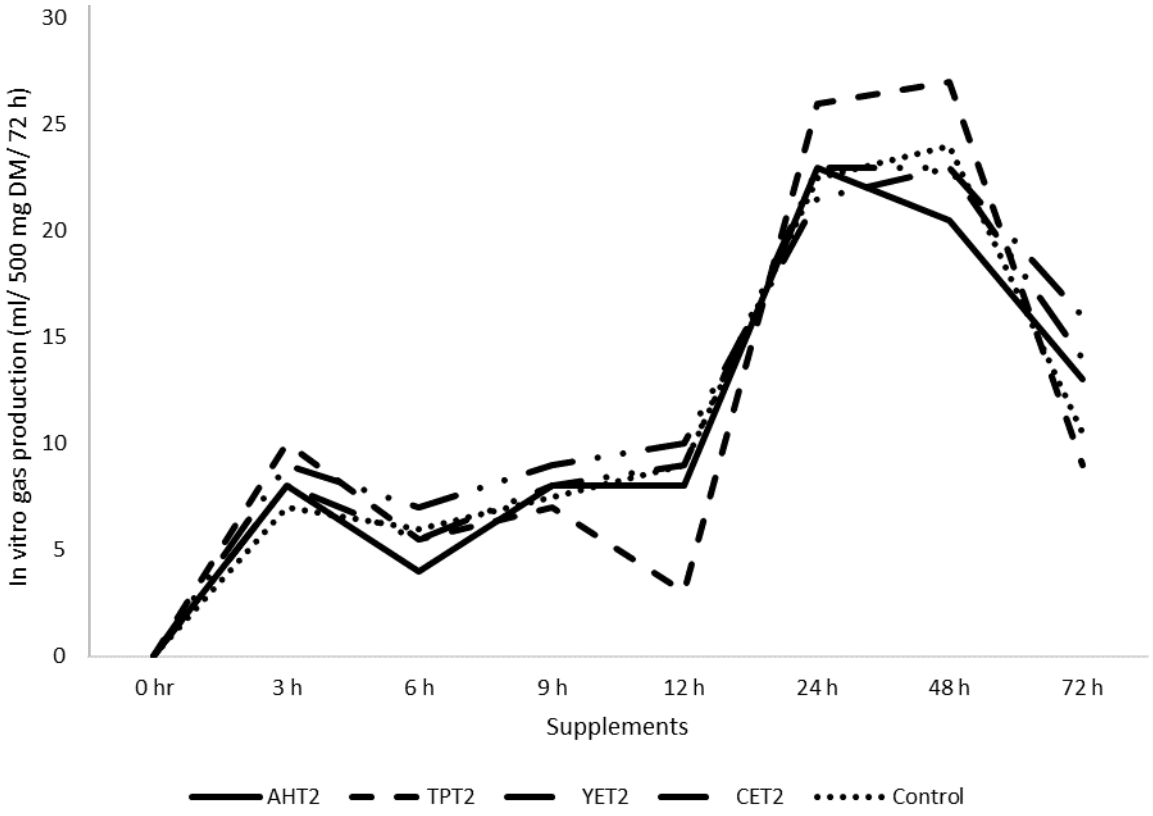


**Figure 1**. *In vitro* gas production of higher level of supplement of each supplement over 72 hours

Some studies have indicated that removal of the protozoal population from the rumen (defaunation) may lower the amount of hydrogen in the system and thereby reduce methane production. Methane production can be decreased by 61% in defaunated rumen fluid (Iqbal et al. 2008). The findings of above researches suggest that the reduced ruminal protozoa population may tends to reduce the methane production. Hence the significantly reduced protozoan count in the present study provides a strong indicator of possible suppression of enteric methane production though it was not measured in the present study.

## CONCLUSION

It can be concluded that the supplementation of guinea grass (*Panicum maximum*) with *Artocarpus heterophyllus* (Jak leaves) *Tridax procumbens*, cellulase enzyme and yeast significantly enhanced *in vitro* total gas production and significantly suppressed the protozoan count. Rumen ammonia nitrogen production was not influenced upon supplementations and in some instances *in vitro* rumen dry matter disappearance and neutral detergent fiber disappearance were increased significantly.

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# Traits of Sheep and Effects of Protein Supplements on Semen Profile in Indigenous Sheep of Bangladesh

Hossain MI[1], Khan MKI[1], Momin MM[1], Miah G[1], Quader MN[2], Miazi OF[1]
[1]*Department of Genetics and Animal Breeding, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh*
[2]*Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh*
*E-mail:* kkhan@cvasu.ac.bd

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**ABSTRAK**

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Penelitian ini dilakukan di distrik Chittagong di Bangladesh dengan kuesioner yang dirancang dengan baik dan terstruktur untuk mengetahui informasi awal tentang domba asli dan efek suplemen protein pada kesuburan. Tiga macam ransum yang mengandung kadar iso-kalori tetapi berbeda dipasok ke tiga kelompok domba yang berbeda di tiga lokasi. Sifat-sifat morfometrik domba seperti panjang rambut, panjang telinga, panjang ekor, panjang badan dan sifat kuantitatif, berat badan di lokasi 3 lebih tinggi daripada dua lokasi lainnya. Panjang rambut jantan (1,91 ± 0,01 cm) lebih besar dari betina sedangkan panjang tubuh rata-rata, panjang ekor dan berat badan betina lebih tinggi daripada jantan. Semua nilai korelasi positif, dimana nilai tertinggi diamati di antara berat badan, panjang tubuh dan tinggi pada pundak (r = 0,73) dan nilai terendah diamati diantara ketebalan dada dan panjang telinga (r = 0,25). Mempertimbangkan persentase sifat-sifat kualitatif dari warna bulu polos, warna kulit tidak berpigmen, warna bulu coklat dan telinga semi-terjumbai ditemukan maksimum daripada yang lain dan nilainya masing-masing 54,21%, 69,16%, 45,79%, 45,79%, 57,01%. Volume semen, jumlah sperma, persentase sperma normal dan viable lebih tinggi dalam pengobatan 2 daripada dua kelompok lainnya. Penelitian ini menyimpulkan bahwa ada pengaruh suplementasi protein pada kinerja reproduksi terutama profil semen dalam ramand studi ini akan menciptakan cakrawala baru produksi domba di Bangladesh.

**Kata Kunci:** Domba, Sifat, Suplemen protein, Kualitas semen

**ABSTRACT**

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The study was carried out at Chittagong district of Bangladesh with a predesigned well-structured questionnaire to know the baseline information of indigenous sheep and effects of protein supplementations on fertility. Three iso-caloric but different graded levels of protein containing rations were supplied to the three different groups of sheep in three locations. The morphometric traits of sheep such as hair length, ear length, tail length, body length and quantitative trait, body weight in the location 3were higher than the other two locations. Hair length of male (1.91±0.01cm) was longer than female whereas the average body length, tail length and body weight of females were higher than the males. All the correlation values was positive, where the highest value was observed among the body weight, body length and withers height (r=0.73) and the lowest value was observed in between chest girth and ear length (r=0.25). Considering the qualitative traits percentage of plain coat color, non-pigmented skin color, brown coat color and semi-pendulous ear found maximum than others and the values were 54.21%, 69.16%, 45.79%, 57.01%, respectively. The semen volume, sperm counts, percentages of normal and viable sperm were higher in treatment 2 than the other two groups. The present study concluded that there is an influence of protein supplementation on reproductive performance especially semen profile in ram and this outcome will create a new horizon of sheep production in Bangladesh.

**Key Words:** **S**heep, Traits, Protein supplements, Semen quality

## INTRODUCTION

In Bangladesh, the sheep are mainly non-descriptive type with their average body weight (15 to 25 kg) and probably originated from south-eastern to sub-tropical regions, having adaptive capacity to hot and humid climate. The common characteristics of indigenous sheep of this country are: grey coat with black or white patches and the face, ear and feet are mostly light black with coarse wool. Sheep are generally sparsely distributed throughout the country and most commonly found in the Rajshahi, Dinajpur, Bogra, Rangpur,

Tangail districts and in the delta regions of Noakhali areas (Bindon 1984) managed under traditional production system. Male fertility is the capability to produce good quality semen and the ability to cause pregnancy in a fertile female which can be measured by the serving capacity test. Semen is the combined secretion of male reproductive glands, which plays a major role in determining the fertility and reproductive efficiency. Rekik et al. (2007) reported that protein has a positive effect on reproductive parameters in case of ram. In ruminants low planes of nutrition during the pre-pubertal period delay testicular growth and the onset of puberty by inhibiting the development of a mature reproductive endocrine system (Brown 1994). The previous study (Zohara et al. 2014) has reported the importance of protein supplementation on growth and productivity of small ruminants. On the other hand, it has long been established that rams reared on higher levels of feeding grow faster and attain puberty at younger ages than rams on lower planes of nutrition (Fernández et al. 2005). Spermatogenesis and production of total number of spermatozoa per ejaculate in rams are responsive to appropriate nutritional management (Fernández et al. 2005). This effect has been related to an increase in testicular size results an increase in the volume of the seminiferous epithelium and in the diameter of seminiferous tubules (Al-kawmani et al. 2014). Undeniable, protein supplements using the concept in sheep ration will help to open a new horizon in the countable improvement of the semen quality and reproductive efficiency. This exploration will be subsidiary for realizing the effects of feed supplements (protein) on semen quality in breeding and selection programme. For this purpose, the study was carried out to evaluate different traits of indigenous sheep of Bangladesh, and to know the effects of protein supplements on semen quantity and quality.

## MATERIALS AND METHODS

The study was carried out at the two upazilla (sub-district) in the Chittagong district of Bangladesh during the month of January 2017 to December 2017.

### Experimental animal selection, ration formulation and study design

A baseline survey was conducted in the studied area with a predesigned questionnaire. A total of 216 sheep, from four different sub-districts (Sandwip 108, Modonhat 16, Hathazarisadar 72 and Bakolia 16) were surveyed directly. The phenotypic and morphological features of the sheep were recorded. Thirty-six indigenous sheep were selected from three different areas (Modonhat (location 1), Hathazari sadar (location 2) and Bakolia (location 3)) from these sub-district on the basis of their body condition score (BCS), health status and normal clinical conditions. Then the sheep were randomly divided into three treatment groups according to location which was heterogeneous in nature and ages were ranged from one to five years. The three groups were managed under the semi-intensive system, kept in separate pens and fed individually according to the group pattern. They were acclimatized and observed regularly for two weeks with a view to screening haemoparasites and helminthes by the farmers and the researcher. Three iso-caloric rations (12 MJ/kg DM) containing graded level of protein were formulated (11.68% CP for control/To, 12.95% CP for T1 and 13.96% CP for T2) by using of conventional feedstuffs (Table 1) and concentrate mixtures (PRO-PAK) were supplied (0.5 kg/day/sheep) to all the groups of sheep for 9 month (March 2018 to November 2018) . Protein-concentrate was provided in both T1 and T2 groups, but there was no protein concentrate in the formulated ration of the control group. All of the treatment groups' sheep allowed for grazing about 8 to 9 hours per day in the grazing land.

### Semen collection and evaluation

At the mid of the trial, all rams under different groups were subjected to a fertility test. Scrotal circumference (SC) was recorded by spermatic cord grasping in cm for find out the correlation with semen volume. The rams were trained for semen collection by artificial vagina (AV) method using receptive restrained ewes. During the trial period, semen collection was performed several times in order to assess semen quality (physical, biochemical and microscopic test). Collected semen samples were evaluated by following the procedure of Zemjanis (1970). The volume of semen was obtained directly from the calibrated tube and recorded. Microscopic examination for wave pattern (gross sperm motility) was determined by placing a drop of raw undiluted semen on a pre-warmed slide and viewed using a field microscope at 40X magnification (Jibril et al. 2011). Sperm concentration was measured by Neubauer-haemocytometer according to the method of Organization (2010). Live and dead ratio of the sperm cells was determined as described by Esteso et al. (2006). A thin smear of the semen sample was made on clean grease free glass slide and stained with eosin-nigrosin stain for enumeration of live dead percentage. Sperm abnormalities were determined by making a thin smear of the semen sample on clean grease free glass slide and fixed with buffered normal saline. In both cases, three hundred thirty-three sperm cells were counted per slide using light microscopy at 40X magnification (Jibril et al. 2011).

**Table 1.**Ingredients And Chemical Composition Of The Experimental Ration

| | Treatment groups | | |
|---|---|---|---|
| | Control(T0) | Treatment 1(T1) | Treatment 2(T2) |
| Ingredients | | | |
| Animals number | 09 | 16 | 11 |
| Maize | 59 | 56.5 | 54.5 |
| Rice Polish | 22 | 22 | 22 |
| Wheat bran | 10 | 10 | 10 |
| Soybean oil | 0.75 | 0.75 | 0.75 |
| Soybean meal | 5 | 5 | 5 |
| Protein concentrate | 0 | 2.5 | 4.5 |
| Dicalcium phosphate | 1.15 | 1.15 | 1.15 |
| DL-Methionine | 0.6 | 0.6 | 0.6 |
| Vitamin B premix | 1 | 1 | 1 |
| Common salt | 0.5 | | 0.5 |
| Calculated chemicals composition | | | |
| Total amount | 100 | 100 | 100 |
| Total Crude Protein (%) | 11.68 | 12.94 | 13.96 |
| Total Crude Fiber (%) | 4.44 | 4.46 | 4.48 |
| Ether Extract (%) | 6.03 | 6.19 | 6.32 |
| Total Energy, ME (MJ) | 12.00 | 12.00 | 12.00 |

**Statistical analysis**

Data was recorded for the phenotypic traits, semen motility, and live dead ratio of sperm cell. The collected data were corrected and analyzed using PROC GLM of SAS (SAS 2008) followed by completely randomized design (CRD). The mean differences were compared using the least significant difference (LSD) at 5% level of significance.

## RESULTS AND DISCUSION

**Quantitative traits of sheep**

The average of phenotypic characteristics (quantitative traits) of sheep under the three different locations is shown in Table 2. Among the locations, the averages of hair length, ear length, tail length, body length and body weight were found comparatively higher in location 3 than the other two locations.

Considering sex, significant differences were observed between sexes for all the traits except the hair length. Hair length was longer in male than female whereas the average body length, tail length and body weights were superior in females than the males.

**The Pearson's correlations among the phenotypic traits (quantitative traits)**

Correlations coefficients (r) of different quantitative traits of the indigenous sheep are shown in Table 3. All the traits are positively correlated with each other ($P < 0.001$). The highest positive correlation was found among the body weight, body length and withers height ($r=0.73$) whereas lowest (positive) correlation was observed in between chest girth and ear length ($r=0.25$) of the studied indigenous sheep population.

**Qualitative traits of sheep**

The qualitative traits of studied indigenous sheep of Bangladesh are shown in Table 4. Irrespective to age and sex, the number of plain coat color sheep was superior (54.21%) than patchy coat color sheep. In the case of skin color, the numbers of non-pigmented sheep were comparatively higher than the pigmented, which was 69.16%. Among the coat color types, brown coat color was found to be highest (45.79%) than the other coat color individuals. Semi pendulous ear was maximum (57.01%) within three areas among the ear patterns. Most of the sheep were polled type, which was 86.92%. Among the tail type patterns, thin tail type nearly 72.90% which was more than fat and fat rump tail percentage.

**Seminal traits**

The mean and standard error of mean (SEM) values of different seminal traits are shown in Table 5. The semen volume was statistically significant ($P<0.05$) among the three treatment groups of ram. The semen volume was higher in T2 which was 0.94 ml than the other two groups. In the case of sperm count T2 contains $4.25\times10^6$sperm, which was higher than the other two groups and SEM value was 0.018 that's consoled those groups were significantly different. Among the three treatment groups, scrotal diameter was found to be larger in T2 than the other two groups which were not significantly different ($P>0.05$) among treatments.

**Table 2.** Mean ± standard error of quantitative traits of sheep of Bangladesh

| Categories | | Traits (average) | | | | |
|---|---|---|---|---|---|---|
| | | Body weight (kg) | Body length (cm) | Ear length (cm) | Tail length (cm) | Hair length (cm) |
| Location | Location 1 | $16.07^{ab} \pm 0.13$ | $79.47 \pm 0.35$ | $9.21^{b} \pm 0.05$ | $10.77^{b} \pm 0.06$ | $1.88 \pm 0.01$ |
| | Location 2 | $13.27^{b} \pm 0.10$ | $77.18 \pm 0.27$ | $12.51^{a} \pm 0.04$ | $12.63^{a} \pm 0.04$ | $1.85 \pm 0.28$ |
| | Location 3 | $19.1^{a} \pm 0.74$ | $86.99 \pm 1.91$ | $12.92^{a} \pm 0.29$ | $12.79^{a} \pm 0.33$ | $2.03 \pm 0.04$ |
| | P-Value | 0.02 | 0.24 | 0.001 | 0.01 | 0.49 |
| Sex | Male | $10.42^{b} \pm 0.16$ | $72.54^{b} \pm 0.43$ | $10.12^{b} \pm 0.06$ | $11.07^{b} \pm 0.07$ | $1.91 \pm 0.01$ |
| | Female | $17.01^{a} \pm 0.08$ | $81.93^{a} \pm 0.10$ | $11.7^{a} \pm 0.08$ | $12.28^{a} \pm 0.03$ | $1.86 \pm 0.01$ |
| | P-Value | 0.001 | 0.003 | 0.05 | 0.01 | 0.43 |

Means with different superscripts i n the same column differ significantly ($p<0.05$)

**Table 3.** Correlations among the phenotypic traits (quantitative traits) of indigenous sheep of Bangladesh

| | Body weight | Body length | Withers height | Chest girth | Ear length | Tail length |
|---|---|---|---|---|---|---|
| Body weight | 1 | 0.73<br><0.001 | 0.73<br><0.001 | 0.71<br><0.001 | 0.35<br><0.001 | 0.36<br><0.001 |
| Body length | | 1 | 0.61<br><0.001 | 0.47<br><0.001 | 0.38<br><0.001 | 0.33<br><0.001 |
| Withers height | | | 1 | 0.53<br><0.001 | 0.48<br><0.001 | 0.49<br><0.001 |
| Chest girth | | | | 1 | 0.25<br><0.001 | 0.26<br><0.001 |
| Ear length | | | | | 1 | 0.42<br><0.001 |
| Tail length | | | | | | 1 |

**Sperm morphology and viability**

Percentages of sperm morphology are shown in Figure 1. Microscopic view of normal and abnormal sperm in Picture 1 whereas live and dead sperm in Picture 2. In Figure 1 percentage of normal sperm was highly assiduous in case of treatment groups (T1, 14.50% sperm was abnormal in nature in case of control, which was higher than the other two groups (Picture 1). Among the three groups, these values have no significantly difference (P>0.05). Sperm viability was not statistically significant among the three groups where T2 contain 87.33% viable sperm, which was higher than the other two groups.

**Table 4.** Qualitative traits of indigenous sheep of Bangladesh

| Traits | Types | Percentage(number) |
|---|---|---|
| Coat color | Plain | 54.21%(58) |
| | Patchy | 45.79% (49) |
| Skin color | Non pigmented | 69.16% (74) |
| | Pigmented | 30.84% (33) |
| Presence of horn | Present | 13.08% (14) |
| | Absent | 86.92% (93) |
| Coat color type | White | 28.97% (31) |
| | Black | 1.86% (2) |
| | Fawn | 14.95% (16) |
| | Brown | 45.79% (49) |
| | Others | 8.41% (9) |
| Ear pattern | Erect | 27.10% (29) |
| | Semi pendulous | 57.01% (61) |
| | Pendulous | 15.89% (17) |
| Tail type | Thin | 72.90% (78) |
| | Fat rump | 9.35(10) |
| | Fat | 17.76% (19) |

**Table 5.** Various seminal traits of ram

| Traits | T0/Control | Treatment 1 (T1) | Treatment 2 (T2) | SEM | P-value |
|---|---|---|---|---|---|
| Semen Volume (ml) | $0.81^{b}$ | $0.88^{ab}$ | $0.94^{a}$ | 0.013 | 0.031 |
| Sperm Count ($10^9$) | $4.09^{b}$ | $4.15^{ab}$ | $4.25^{a}$ | 0.018 | 0.04 |
| pH | $7.43^{a}$ | $7.07^{b}$ | $6.95^{b}$ | 0.028 | 0.002 |
| Scrotal diameter(cm) | 13.10 | 17.86 | 20.82 | 2.248 | 0.225 |

Means with different superscripts in the same row differ significantly among the treatment groups (P<0.05)

***Phenotypic characteristics (Quantitative traits) of Sheep***

Phenotypic characteristics of indigenous sheep population showed the presence of clear morphological variations according to production systems diversity persisted in indigenous sheep. A similar finding was reported by Yakubu et al. (2011) with the current study.

The average body weight of sheep under this investigation was found similar to the results of Nsoso

et al. (2004) and Pervage et al. (2012). The female sheep attained more live weight which is also matched with Nsoso et al. (2004) who reported the body weight of indigenous sheep was higher in the female than the male. Differences of body length were observed between male and female sheep which was higher than the findings of Hassan & Talukder (2012) and conform to the study of Pervage et al. (2012) in the case of indigenous sheep.

The body length of indigenous sheep was varied, and this variation in body length occurs due to the differences in genetic make-up, feeding management, housing management, health and other management factors and same factors was described by Hassan & Talukder (2012) for the body length variation of sheep.Ear length of indigenous sheep firmly coincided with the findings of Nsoso et al. (2004) and larger than the findings of Pervage et al. (2012) and Hassan & Talukder (2012). This variation in the measurements might be due to the variation in different managerial system and ages of sheep. Average tail length of sheep was similar to the measurement of Hassan & Talukder (2012) and Nsoso et al. (2004),they found that the tail length was 12 cm in indigenous sheep. Tail length could be varied due to differences in production systems among the studied indigenous sheep.

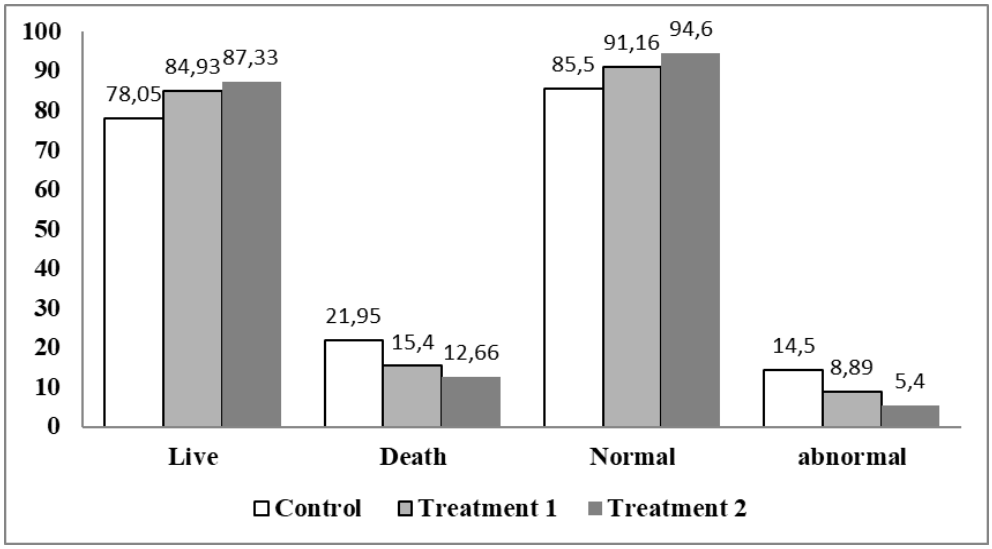
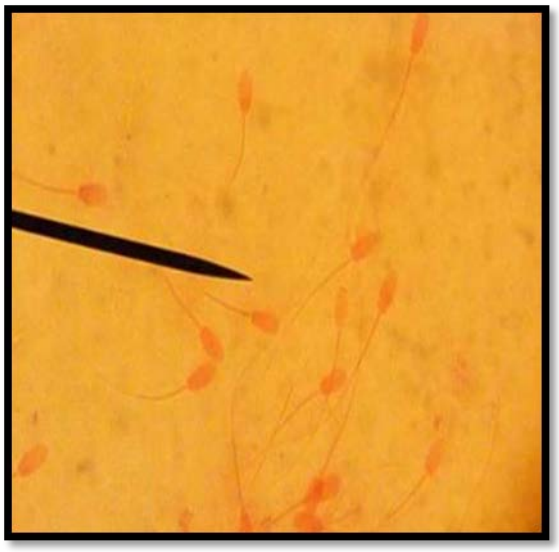



**Figure 1.** Percentage of sperm morphology



**Picture 2 :** Normal and abnormal sperm

**Picture 3:** Live and dead sperm

**Seminal traits and sperm morphology**

Reproductive performance of livestock is determined by various factors those are genetic merit, physical environment, nutrition and management. Nutritional factors are perhaps the most crucial in terms of their direct effects on the reproductive phenomenon and the potential to moderate the effect of other factors. There was an interrelationship between energy intake and reproductive performance in adult rams (Kheradmand et al. 2006; Jibril et al. 2011).

Semen volume is one of the important factors in semen evaluation and reproduction performance in the males (Ax et al. 2016). The semen volume was0.94 ml in Treatment 2 group (protein-rich feed) which was harmonious with the findings of Fernández et al. (2005), Abera et al. (2014) and Asefa et al. (2017)where they denoted that control group generates less volume semen than the treatment groups. On the other hand, there was in inconsistent with the findings of Jibril et al. (2011) who said that semen volume was not influenced by the level of protein. This variation may occur due to changes in protein percentages in ration and farm management system. Nutrition has effect for the growth and maturation of sertoli cells in newborn lambs, which are strong candidates for future performance because the number of sertoli cells was highly correlated with the maximum rate of sperm production (Bielli et al. 1999) and sperm concentration. In the present experiment sperm count in T2 was higher than the other two groups, strongly coincided with the research outcome of (Jibril et al. 2011) where they said that increase crude protein intake above the minimum requirements resulted in improved sperm concentration and favors for spermatogenesis.

Scrotal diameter, testicular sizes were affected by nutrition, where testicular growth can be affected when animals are fed above their maintenance requirement. The findings of present studies of scrotal diameter were more sizably voluminous in the treatment group 2 than the other two groups which were agreed with the results obtained by Fernández et al. (2005) where they noticed that the improve protein-rich diet was helpful for larger growth of scrotal circumference than the control group, although inconsistent with those obtained by Bielli et al. (1999) who found no significant effect from high dietary protein on testicular dimensions. Percentage of normal sperm was highly diligent in case of treatment groups than the control group. Here percentage of normal sperm are more in the case of protein enriched Treatment 2 group which was consistent with the findings of Kheradmand et al.(2006) and Jibril et al. (2011) and did not matched with the findings of Barth et al. (2008) who found that the medium or high level of nutrition does not have influenced on overall percentage of morphologically normal spermatozoa. Present studies indicated that the sperm viability was not statistically significant among the three groups where Treatment group 2 contains higher viable sperm than the other two groups and strongly coincide with the findings of Jibril et al. (2011).

## CONCLUSION

Indigenous sheep from three different areas were varied phenotypically. All the phenotypic traits were positively correlated with each other. The percentage of plain coat color, non-pigmented skin color, brown coat color and semi-pendulous ear were significantly higher than other qualitative traits. The seminal traits were better in treatment 2 (13.96% CP) than the other two groups that suggest that improved dietary intake above maintenance requirements had positive effects on rams and reproductive performances that presage to rear sheep with on supplements may improve the sheep production in Bangladesh.

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# Meat Quality on Sentul Cocks with Different Immunoglobulin Yolk Concentrations

Ariyanti R[1], Ulupi N[1], Suryati T[1], Arifiantini RI[2]

[1]*Departemen Ilmu Produksi dan Teknologi Peternakan, Fakultas Peternakan, IPB, Bogor.*
[2]*Departemen Klinik, Reproduksi dan Patologi, FKH, IPB, Bogor*
*E-mail:ria_ariyanti@yahoo.co.id*

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**ABSTRAK**

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Ayam jantan Sentul adalah salah satu ayam lokal Indonesia yang awalnya dikembangkan masyarakat Kabupaten Ciamis, Provinsi Jawa Barat. Sel tubuh ayam yang sehat dapat berfungsi dengan baik, terutama dalam proses metabolis. Ayam sehat dapat menghasilkan perkembangan otot yang lebih baik. IgY adalah substansi protein otot yang mampu menetralisir sejumlah mikroorganisme penyebab infeksi. Tujuan dari penelitian ini adalah untuk mengevaluasi efek konsentrasi IgY pada kualitas fisik dan organoleptik daging. Penelitian ini menggunakan 20 ekor ayam jantan berumur 4 bulan dengan 2 perlakuan (konsentrasi IgY di atas 9.30 ± 0.45 mg $mL^{-1}$ dan di bawah 9.30 ± 0.45 mg $mL^{-1}$). Variabel yang diamati ternasuk kualitas fisik dan organoleptik daging yang diuji menggunakan Rancangan Acak lengkap. Data dianalisis menggunkan T-test. Dapat disimpulkan bahwa ayam jantan dengan konsentrasi IgY di atas 9.30 ± 0.45 mg $mL^{-1}$ memproduksi daging dengan melonaldehida lebih rendah. Keberadaan konsentrasi melonaldehida yang rendah dalam daging ini menunjukkan daging yang lebih sehat.

**Kata Kunci**: Ayam Sentul Jantan, IgY, Kualitas Daging

**ABSTRACT**

Ariyanti R, Ulupi N, Suryati T, Arifiantini RI. 2019. Meat Quality on Sentul Cocks with Different Immunoglobulin Yolk Concentrations. JITV 24(2): 76-81. DOI.: http://dx.doi.org/10.14334.jitv.v24i2.1861

Sentul cocks is one of the native chicken breeds in Indonesia which is originally raised by villagers in Ciamis District, West Java. Healthy chicken cells can function properly, especially in the metabolic process. Healthy chickens are expected to produce better muscle development. IgY is a protein molecule substance that can neutralize a number of microorganisms that cause infection. The purpose of this study was to evaluate the effect of IgY concentration on physicochemical and organoleptic qualities of meat. This study used 20 cocks, 4th month ages, consist of 2 treatments (IgY concentrations above 9.30 ± 0.45 mg $mL^{-1}$ and IgY concentrations below 9.30 ± 0.45 mg $mL^{-1}$). The variable observed include physicochemical and organoleptic quality of meat. The study was used completely randomized design. Data were analyzed by t-test. The result concluded that cocks with concentrations above 9.30 ± 0.45 mg $mL^{-1}$ produced meat with lower malonaldehyde. The low content of malonaldehyde in meat shows that the meat produced is healthier.

**Key Words**: Sentul Cocks, IgY, Meat Quality

## INTRODUCTION

Sentul cock is one of the germplasm originating from villagers in Ciamis District, West Java. This chicken is very potential to be commercially bred to fulfill people's nutrition and increase farmers' income (Sulandari et al. 2007). Muhsinin et al. (2016) stated that sentul cock was able to neutralize *S. pullorum* bacteria by 26% -60% through in vitro testing using the method of clearance test with a dose of $10^7$ CFU $mL^{-1}$. This situation proves that the resistance of sentul cocks varies greatly. IgY is a protein molecule substance that can neutralize a number of microorganisms that cause infection. According to Regar et al. (2013) livestock that has high resistance can fight infectious agents so they can produce a good performance.

Sentul cocks have a very large role in producing offspring and increasing the performance of the next generation. Sentul cocks that has high IgY is a chicken that is more resistant to disease attacks and healthier (Setyawati et al. 2019). Wiryawan et al. (2005) stated that broilers given antibacterial (*alicin*) in their feed produced healthy chickens and high body weight. Healthy chicken cells can function properly, especially in the metabolic process. Healthy chickens are expected to produce better muscle development. The importance of IgY concentration in the metabolic process, the purpose of this study was to evaluate the quality of sentul cocks meat that has different IgY concentrations.

## MATERIALS AND METHODS

### Cocks Research

The cocks used were 20 sentul cocks, 4 months old. Cocks body weight at the start of the study was around 1.6-2.2 kg bird$^{-1}$ with an average of 1.9 kg bird$^{1}$.

### Testing the IgY concentration of Sentul cocks

Testing of IgY concentrations was carried out on 20 sentul cocks. Testing of total IgY in blood serum was carried out using the indirect method of ELISA (Enzyme-Linked Immunosorbent Assay) according to Yokoi et al. (2002). Cocks that have IgY concentrations above the average are classified as chickens with high IgY concentrations. Cocks that have an IgY concentration equal to or below the average are classified as cock with low IgY concentrations.

### Maintenance of cocks

Sentul cocks were maintained during the pre-layer and layer to determine their production performance. The feed used was commercial phase layer crumble (protein 16-18% and metabolic energy 2700-2800 kcal kg$^{-1}$). The provision of feed and drinking water was carried out adlibitum on the morning at 07.00 WIB and in the afternoon at 16.00 WIB. Cocks slaughter was carried out at the end of maintenance, 28 weeks.

### Cocks Slaughter

Cocks slaughter was carried out at the end of maintenance for observing physicochemical variables of meat. Cocks were fasted for 12 hours before slaughter (Sandi et al. 2012). Slaughter was done lawfully in accordance with CAC / GL 24-1997 (BSN 2009), namely by cutting the neck (carotid artery, jugular, tracheal and oesophageal veins).

### Physicochemical Quality Test of cocks Meat

Physicochemical quality testing of cocks meat in this study included pH value, the percentage of free $H_2O$, cooking shrinkage, tenderness, aw value and the content of malonaldehyde meat. The high content of malonaldehyde in meat shows that the meat produced is not healthy because it undergoes an oxidation process.

Potential Hydrogen (pH) was measured using a meat pH meter (AOAC 2005). The calibrated pH meter was then inserted into the meat sample and left until the numbers printed on the digital measurements did not change. The percentage of free $H_2O$ was analyzed by the Hamm (1972) in Soeparno (2005) using a carper press. 0.3 g of meat was placed on the carper press and the maximum pressure was applied with a load of 35 kg for 5 minutes until a liquid circle (outer circle) and outer circle of meat (inner circle) were formed, then calculation outside the wet area using a planimeter. The percentage of free $H_2O$ was analyzed by the Hamm (1972) in Soeparno (2005) using a carper press. Processing steps as follows, (1) 0.3 g of meat was placed on the carper press and (2) the maximum pressure was applied with a load of 35 kg for 5 minutes until a liquid circle (outer circle) and outer circle of meat (inner circle) were formed, (3) then calculation outside the wet area using a planimeter and (4) the water content that came out of the meat after the emphasis, was calculated by the following formula. The calculation outside the wet area was calculated by the outer circle minus the circumference and divided by one hundred, calculation mg $H_2O$ with the wet area (cm$^2$) divided by 0.0948 minus 0.8 and the percentage of water that comes out is calculated with mg $H_2O$ divided by 0.3 g then multiplied by one hundred percent.

Water activity ($a_w$) was measured using the Salejda et al. (2014) procedure using $a_w$ meter. The mashed sample was put into the sample chamber at aw meter, then the sample was left to stand for approximately 15 minutes. The constant $a_w$ value was then recorded. Cooking loss was analyzed using the Tijare et al. (2016) by weighing meat as much as 100 g. The sample was put in boiling water until the meat temperature reached 76$^0$C (about 10 minutes) and was measured using a bimetal thermometer. The measurement of the percentage of cooking shrinkage was done by reducing the weight before and after cooking / draining divided by the initial weight. Tenderness was measured according to the procedure of Bowker et al. (2014) using the Warner-Bratzler shear force tool. Meat samples were pierced with a bimetal thermometer, boiled in boiling water until the internal temperature was 76$^0$C. After that, the meat sample was cooled for 60 minutes. The meat was formed in the same direction as the 1 cm$^2$ meat fiber and then measured by the WB tool and the meat tenderness would be read on the scale of the tool.

MDA is a marker for the formation free radicals of meat (Nielsen et al. 1997). The content of malonaldehyde (MDA) was measured using the (Nielsen et al. 1997) method modified by Suryati et al. (2013). Meat samples were added with 100 mL of distillate water containing 0.1% propyl galate (PG) and 0.1% ethylene diamin tetra acetate (EDTA) and stirred until smooth. The mixture was then transferred quantitatively into a distillation tube through washing with the addition of 97.5 mL of distillate water containing 0.1% PG and 0.1% EDTA. The mixture was acidified with 2.5 mL of hydrochloric acid (HCl) solution with a ratio of 1: 2 (HCl: aquades) and added 5 drops of anti-froth A (Sigma Aldrich USA). Distillation

was carried out to obtain 50 mL of distillate for each sample. Determination of thiobarbituric acid reactive substance (TBARS) was carried out using a spectrophotometer at λ 532 nm. A total of 5 mL of sample distillate was mixed with 5 ml of 0.02 M TBA solution (Sigma Aldrich USA) in a glass tube, and then incubated at 100 o C water baths for 40 minutes before being cooled to room temperature and running water. All sample distillates were analyzed in duplicate. The calibration curve was made from a solution of 4 stock 1,1,3,3-tetra etioxy propane (TEP) (Sigma Aldrich USA) 0.002 M which was reacted with a TBA solution and treated the same as a sample. The standard curve is made from the relationship between absorbance at λ 532 nm with the concentration of TEP or MDA. Numbers of TBARS are expressed as mg MDA per kg of sample. The MDA calculation is done by the formula:

$$\text{MDA grade} = \frac{C_{MDA} \times V_{des}}{M_S}$$

Information :

$C_{MDA}$ : MDA concentration as read on the standard curve

$V_{des}$ : volume of distillate (mL)

$M_S$ : weight (massa) sample (g)

**Organoleptic Test**

Observations were carried out according to the method (Smith et al. 2012) by cutting meat in the chest size 3 $cm^3$ and tested in raw and mature conditions on 30 panelists consisting of undergraduate and postgraduate students using IPB questionnaires. The test was in the form of a hedonic test (level of preference which includes color, texture, and aroma) and hedonic quality (level of product quality). In this test, panelists were asked to give an assessment with a score of 1-5.

**Data analysis**

The design was used a completely randomized design (CRD) with two treatments. The treatment is the different serum (high and low) IgY concentrations. Each treatment was repeated 10 times. Each treatment was repeated ten times. Each replication consists of 1 Sentul cocks. The variables observed were meat quality (physicochemical and organoleptic). The data obtained were analyzed by t-test using Minitab program (Mattjik & Sumertajaya 2013) with the following formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$$

Information:

$n_1$ : number of observations of meat quality with IgY levels above 9.30 ± 0.45 mg $mL^{-1}$

$n_2$ : number of observations of meat quality with IgY levels below 9.30 ± 0.45 mg $mL^{-1}$

$x_1$ : average meat sample with IgY level above 9.30 ± 0.45 mg $mL^{-1}$

$x_2$ : average meat sample with IgY level below 9.30 ± 0.45 mg $mL^{-1}$

$s_1$ : standard deviation of meat with IgY level above 9.30 ± 0.45 mg $mL^{-1}$

$s_2$ : standard deviation of meat with IgY levels below 9.30 ± 0.45 mg $mL^{-1}$

## RESULTS AND DISCUSION

***Physicochemical Quality of Meat***

The results of the physicochemical quality observations of sentul cock meat in this study are presented in Table 1. Based on statistical tests it was found that the pH value, percentage of free $H_2O$, $a_w$, cooking loss, and tenderness were not statistically significant different, but were significantly different in the malonaldehyde content of breast meat. Sentul cocks that had a high IgY concentration actually produce lower meat malonaldehyde content. The low MDA value indicates that meat is included in the category of health because it does not undergo the process of fat oxidation.

Potential Hydrogen (pH) of sentul cocks (chest part) of this study turned out to be lower than the results of the Khaerunnisa et al. (2016). This is caused by the chicken body weight used is different. Cocks with higher body weight have higher glycogen content so that higher levels of rigor mortis are produced (Pragati et al. 2007).

The cooking loss value of sentul cocks meat in this study amounted to 20.44% -23.06%. These results are better than the value of cooking losses of village cocks at the same age of 28 weeks that is equal to 41.06%-41.50% (Khaerunnisa et al. 2016). The tenderness of cocks boiled breast meat in this study was 2.82-3.41 kg $cm^{-2}$. According to Bowker et al. (2014), this value is still in the soft category. The aw value produced in this study using sentul cocks meat is lower than the research conducted by Aberle et al. (2001) which uses broiler chicken meat. The higher the aw value, the more water

**Table 1.** Physicochemical quality of sentul cock meat with different IgY concentrations

| Variable | IgY concentration below 9.30 ± 0.45 mg $mL^{-1}$ | IgY concentration above 9.30 ± 0.45 mg $mL^{-1}$ |
|---|---|---|
| pH value | 5.11±0.06 | 5.16±0.06 |
| Free of $H_2O$ (%) | 30.42±4.10 | 31.33±6.00 |
| Cooking loss (%) | 23.06±0.08 | 20.44±0.07 |
| Tenderness (kg $cm^{-2}$) | 3.41±1.06 | 2.82±1.05 |
| $a_w$ value | 0.90±0.02 | 0.90±0.01 |
| Malonaldehyde content (MDA, mg $kg^{-1}$) | 1.20±0.16a[1)] | 0.71±0.15b |

Different letters on the same line show significant differences (P<0.05).

activity in the myofibrils, so the bacteria will grow faster and multiply in the flesh (Troller & Christian 1978). This means that bacteria are more difficult to live in sentul cocks (28 weeks) than broiler chicken with an age of about 5 weeks.

Sentul cocks which have low IgY has low lymphocytes in the blood (Setyawati et al. 2019). Lymphocytes are one of the leukocytes that function to form IgY or antibodies. Lymphocyte concentration in chickens is one indicator of heat resistance. Low lymphocyte value will increase the ratio of heterophils and lymphocytes (H/L). This increase in the ratio indicates that chickens are experiencing stress due to the heat of the maintenance environment (Munck et al. 1984; Davis et al. 2008). The temperature of maintenance of chicken's cages in this study ranged from 24-31 $^{o}$C. This condition causes a high-fat oxidation reaction. Increasing the oxidation reaction will produce high free radicals, so the MDA content produced is also high (1.20 mg $kg^{-1}$). Chickens that have high IgY concentrations in this study produce meat with a lower MDA content (0.71 mg $kg^{-1}$). However, the high MDA content in meat originating from sentul cocks with low IgY in this study is still lower than the standard MDA content of food, which is 1.8 mg $kg^{-1}$ (Gray 1978). Meat that has sub-standard MDA content is healthy meat. The low MDA value indicates that meat does not undergo a process of fat oxidation which causes free radicals. Foods that experience free radicals can stimulate cancer cell growth.

### Organoleptic Quality

The observations of the organoleptic quality of sentul cocks breast meat in this study are presented in Table 2. Based on statistical tests it was found that the color, texture, and aroma of sentul cocks meat with different IgY concentrations did not show any difference in both hedonic and hedonic quality. Based on the panelist's assessment of hedonic quality (product quality), sentul cocks meat in this study was slightly pale in color, textured rather soft and slightly fishy scented. The color of the meat is influenced by the myoglobin content of meat. Myoglobin is a color pigment that resides in meat. The redder the color of the meat, the higher the concentration of pigments in the meat (Ladikos & Wedzicha 1988). Organoleptic tests using raw chicken and observations about the texture using raw and cooked meat.

Hedonic quality (product quality based on panelist assessment); color brightness: 1: very pale, 2: pale, 3: slightly pale, 4: slightly bright reddish, 5: bright reddish; hardness level of texture 1: very hard, 2: hard, 3: slightly soft, 4: soft, 5: very soft; fishy aroma level 1: very fishy, 2: fishy, 3: fishy, 4: not fishy, 5: very fishy. Hedonic (panelist's favorite level): 1: dislike, 2: rather like, 3: like, 4: really like, 5: really like it very much.

Hedonic panelists' assessment (likes) shows that the panelists consisting of post-graduate students rather like the color, texture, and aroma of sentul cocks meat. This is because panelists are accustomed to seeing broiler meat that has the color, texture, and aroma of meat that is quite different when compared to local chicken meat.

## CONCLUSION

Sentul cocks with IgY concentrations above 9.30 ± 0.45 mg $mL^{-1}$ were able to produce lower malonaldehyde content, so that the meat produced is healthier.

**Table 2**. Organoleptic (hedonic and hedonic quality) qualities of cock's meat with different IgY concentrations

| Testing | Raw meat | | Cooked meat | |
|---|---|---|---|---|
| | A | B | C | D |
| Hedonic quality | | | | |
| Color | 2.60±0.85 | 2.76±0.89 | - | - |
| Texture | 3.27±0.83 | 3.53±0.78 | 3.00±0.87 | 3.07±0.79 |
| Aroma | 3.17±0.91 | 3.30±0.79 | - | - |
| Hedonic | | | | |
| Color | 2.37±0.99 | 2.67±0.80 | - | - |
| Texture | 2.83±1.02 | 2.90±0.89 | 2.63±1.00 | 2.70±0.70 |
| Aroma | 2.70±1.06 | 2.87±0.90 | - | - |

A = IgY concentration below 9.30 ± 0.45 mg $mL^{-1}$
B = IgY concentration above 9.30 ± 0.45 mg $mL^{-1}$
C = IgY concentration below 9.30 ± 0.45 mg $mL^{-1}$
D = IgY concentration above 9.30 ± 0.45 mg $mL^{-1}$

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e-mail: jitvnak@yahoo.com/jitvnak@litbang.pertanian.go.id
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