ISSN 0853-7380
E-ISSN 2252-696X

Accredited by the Ministry of Research and Technology /
National Agency for Research and Innovation
Decree Number: 85/M/KPT/2020

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 25
Number 4
December 2020

PUSAT PENELITIAN DAN PENGEMBANGAN PETERNAKAN
BADAN PENELITIAN DAN PENGEMBANGAN PERTANIAN
KEMENTERIAN PERTANIAN

JITV	Volume 25	Number 4	Page: 147-218	Bogor, December 2020	ISSN 0853-7380

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Published by:
Indonesian Center for Animal Research and Development
Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture

Collaborated with:
Indonesian Society of Animal Science

Secretariat of IJAVS:
Jalan Raya Pajajaran Kav. E-59, Bogor 16128 - Indonesia
Telephone +62-251- 8322185
Fax +62-251-8380588
E-mail: criansci@indo.net.id; jitvnak@yahoo.com
Website: http://medpub.litbang.pertanian.go.id/index.php/jitv

Indonesian Journal of Animal and Veterinary Sciences is published four times a year in March, June, September and December.

PREFACE

In this edition, Volume 25 No 4, we proudly present articles from animal and veterinary sciences including genetics; reproduction, feed technology, and veterinary. The articles published in this edition are:

"Genetic and Non-Genetic Effects on Semen Characteristics of Bali Cattle (*Bos javanicus*)"; "Improving the Effects of Omega-3 Fatty Acid on the *In Vitro* Maturation of Oocytes"; "Reproduction Status and Population Dynamic of Kuantan Cattle in the Kuantan Singingi Regency"; "Novel Mutation of Exon 5 Prolactin Gene in IPB-D1 Chicken"; "Effect of *Averrhoa bilimbi* Fruit Filtrate and Shrimp Paste Mixture on Performance, Gut Microbes and Blood Profile of Broilers, Gut Microbes and Blood Profile of Broilers", "Chemical Quality of Culled Duck Meatball (*Anasplathyryncos*) Substituted with Edamame Flour (*Glycine max* (L) Merril) Filler", and "Effects of Probiotic, Prebiotic, and Synbiotic Mixed Culture Based on Wheat Pollard on Productivity of Kampung's Chicken".

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, December 2020

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Volume 25, Number 4, December 2020 ISSN 0853-7380 E-ISSN 2252-696X

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Genetic and Non-Genetic Effects on Semen Characteristics of Bali Cattle (*Bos javanicus*)

Sitanggang G[1], Arifiantini RI[2], Jakaria J[3]

[1]*Directorate General of Livestock and Animal Health, Ministry of Agriculture, Republic of Indonesia*
Jl. Harsono RM No. 3 Ragunan, Pasar Minggu, Jakarta Selatan 12550, Indonesia
[2]*Faculty of Veterinary Medicine, IPB University, Jl. Agatis, Dramaga Campus, Bogor 16680, Indonesia*
[3]*Department of Animal Production and Technology, Faculty of Animal Science, IPB University*
Jl. Agatis, Dramaga Campus, Bogor 16680, Indonesia
Email: jakaria_karman@yahoo.co.id

(received 20-07-2020; revised 15-08-2020; accepted 22-09-2020)

ABSTRAK

Sitanggang G, Arifiantini RI, Jakaria J. 2020. Pengaruh genetik dan non genetik karakteristik semen sapi Bali (*Bos javanicus*). JITV 25(4): 147-152. DOI: http://dx.doi.org/10.1433/jitv.v25i4.2526

Penelitian ini bertujuan mengevaluasi pengaruh faktor genetik dan non-genetik terhadap karakteristik semen meliputi volume semen, konsentrasi spermatozoa, jumlah total spermatozoa dan motilitas spermatozoa sapi Bali. Data semen (volume semen, konsentrasi spermatozoa, jumlah total spermatozoa dan motilitas spermatozoa) diperoleh dari Balai Besar Inseminasi Buatan Singosari, Malang, Jawa Timur, Indonesia. Sapi yang digunakan berjumlah 17 ekor dengan total 3.847 ejakulat pada tahun 2014 sampai 2016. Data dianalisis dengan metode *restricted maximum likelihood* (REML) menggunakan *mixed model* dengan individu sapi sebagai pengaruh acak, sementara umur, musim, frekuensi ejakulasi dan interval penampungan sebagai pengaruh tetap. Hasil studi menunjukkan bahwa umur berpengaruh nyata (P<0,01) terhadap semua karakteristik semen. Musim hanya berpengaruh terhadap motilitas spermatozoa (P<0,01). Frekuensi ejakulasi dan interval penampungan berpengaruh nyata terhadap semua karakteristik semen (P<0,01), kecuali motilitas spermatozoa. Ripitabilitas volume semen, konsentrasi spermatozoa, jumlah total spermatozoa dan motilitas spermatozoa masing-masing sebesar 0,43; 0,35; 0,32 dan 0,31. Disimpulkan bahwa umur, frekuensi ejakulasi dan interval penampungan sangat berpengaruh terhadap karakteristik semen sapi Bali. Ripitabilitas karakteristik semen termasuk sedang sampai tinggi.

Kata Kunci: Sapi Bali, Non-Genetik, Ripitabilitas, Karakteristik Semen

ABSTRACT

Sitanggang G, Arifiantini RI, Jakaria J. 2020. Genetic and non-genetic effects on semen characteristics of Bali cattle (*Bos javanicus*). JITV 25(4): 147-152. DOI: http://dx.doi.org/10.1433/jitv.v25i4.2526

The objective of this study was to evaluate effect of genetic and non-genetic factors on semen characteristics including ejaculate volume, sperm concentration, total sperm number and sperm motility of Bali cattle. Semen data were collected from the National Centre of Artificial Insemination at Singosari, Malang, East Java, Indonesia. A total of 3,847 ejaculates of 17 Bali bulls from 2014 to 2016 were collected and evaluated. Data were analyzed by restricted maximum likelihood (REML) method using mixed models which the bull was a random effect, while age of bull, season of collection, frequency of ejaculation and collection intervals were the fixed effects. Results showed that age significantly affected all semen characteristics (P<0.01). Season affected only on sperm motility (P<0.01). Effect of frequency of ejaculation and collection intervals was significant on all studied variables (P<0.01), except sperm motility. Repeatability of ejaculate volume, sperm concentration, total sperm number and sperm motility was 0.43; 0.35; 0.32 and 0.31, respectively. It is concluded that age, frequency of ejaculation and collection intervals were the most factors affected semen characteristics of Bali cattle. Repeatability estimations of semen characteristics were moderate to high.

Key Words: Bali Cattle, Non-Genetic, Repeatability, Semen Characteristics

INTRODUCTION

Bali cattle (*Bos javanicus*) is an Indonesian native cattle domesticated from direct descendants of wild Banteng (*Bibos banteng*) (Martojo 2012; Purwantara et al. 2012). The breed had been acknowledged by Food and Agriculture Organization as a world breed (FAO 2007). Bali cattle were spread in almost all Indonesian provinces. In 2011, the population of Bali cattle was recorded at 4.7 million heads (Badan Pusat Statistik 2011) and it represents 32% of the total Indonesian cattle population.

Bali cattle has several potential traits, such as the ability to survive in harsh environments (Panjaitan et al. 2014), high fertility and conception rate (Purwantara et al. 2012), high carcass percentage (Tahuk et al. 2018), and high meat quality (Jakaria et al. 2017). Hence, this breed has the potential for meat production and contributes to fulfill the national beef demand. The Indonesian government strives to increase the population and genetic quality of Bali cattle through artificial insemination (AI). The success or failure of AI is influenced by the quality of semen. The quality of semen itself is affected by genetic and non-genetic factors.

Repeatability evaluation on semen characteristics might be useful for selection of superior bulls. Repeatability estimations of semen characteristics such as ejaculate volume, sperm concentration, total sperm number and sperm motility were reported to vary from moderate (Karoui et al. 2011), high (Burren et al. 2019), and moderate to high (Atagi et al. 2017). The effect of non-genetic factors on semen characteristics is important to improve semen characteristics. Studies regarding the effect of non-genetic factors such as age of bull, season of collection, frequency of ejaculation and collection intervals on semen characteristics vary in different breeds and countries (Boujenane & Boussaq 2013; Snoj et al. 2013; Murphy et al. 2018). However, there is still less information in Bali cattle. Therefore, this study was done to investigate genetic and non-genetic effects influencing semen characteristics in Bali bulls.

MATERIALS AND METHODS

Semen data, animals and location

Data of semen characteristics were collected from 2014 to 2016. Those data were provided by the National Artificial Insemination (AI) Centre at Singosari, Malang, East Java, Indonesia. The AI Center has an ambient temperature between 16 and 22 ^{o}C, with humidity ranging from 70 to 90%. The AI Center was established in 1982.

A total of 3,847 ejaculates from 17 Bali bulls were evaluated. The average age of bulls was in a rank of 65 months, with the youngest of 25 months to the eldest of 171 months. The bulls were similarly maintained on feeding and management during the semen production period.

Semen collection and evaluation

In the AI Centre, semen was routinely collected twice a week at early morning by artificial vagina with the frequency of ejaculation 1-2 times with 15 minutes interval between each ejaculation. The bulls mounted a teaser animal, but a dummy was also available. Ejaculate volume was obtained through scale reading from the graduated collection tube (milliliters). Sperm concentration (10^6 per milliliters) was determined using Photometer SDM 6 (Minitube, Germany). Sperm motility was presented percentage (%) on dilution stage and measured by a microscopic examination with a warm stage at 200 × magnification (Olympus BX 53, Minitube, Germany). Total sperm number were calculated by multiplying of ejaculate volume to sperm concentration.

Statistical analysis

Non-genetic factors

The effect of non-genetic factors on semen characteristics was analyzed using the following generalized linear model (GLM).

$Y_{ijkl} = \mu + A_i + S_j + E_k + I_l + \varepsilon_{ijkl}$

Where, Y_{ijkl} is semen characteristics measured (ejaculate volume, sperm concentration, total sperm number and sperm motility); μ is overall means; A_i is fixed effect of i^{th} age of bull (i = > 2 to 4.5 years = 6 bulls, > 4.5 to 7 years = 6 bulls, > 7 to 9.5 years = 2 bulls, > 9.5 to 12 years = 2 bulls, > 12 to 14.5 years = 1 bull); S_j is fixed effect of j^{th} season of collection (j = rainy: October to March, dry: April to September); E_k is fixed effect of k^{th} frequency of ejaculation (k = first ejaculate, second ejaculate); I_l is fixed effect of l^{th} collection intervals (l = 3 days, 4 days); ε_{ijkl} is random residual effect.

Significant differences among treatment means were tested through Duncan's multiple range test at level of 1% ($P<0.01$).

Repeatability

Repeatability estimations for semen characteristics were calculated by using the following formula.

$$r = \frac{\sigma_s^2}{\sigma_s^2 + \sigma_e^2}$$

Where, r is repeatability; σ_s^2 is the sire variance; σ_e^2 is the error variance.

Repeatability estimations were determined using mixed model analysis where the bull as random effect variables and the fixed effects of age, season, frequency of ejaculation and collection intervals. All statistical analyses were performed using SAS software version 9.4 (SAS Institute 2013).

RESULTS AND DISCUSSION

The Bali bulls had an average ejaculate volume of 4.85 mL, sperm concentration of 990 × 10^6/mL, total

sperm number of 4,759 × 10^6 and sperm motility of 60% (Table 1). Other studies reported that average values were in the range of 2.06-7.22 mL for ejaculate volume, 475-1,312 × 10^6/mL for sperm concentration, 3,023-8,352 × 10^6 for total sperm number and 55-85% for sperm motility (Bhakat et al. 2011; Karoui et al. 2011; Boujenane & Boussaq 2013; Burren et al. 2019; Yin et al. 2019; Olsen et al. 2020).

Non-genetic effect

Table 2 presents least square means along with their standard errors for ejaculate volume, sperm concentration, total sperm number and sperm motility of Bali bulls. Non-genetic effects were discussed as belows:

Effect of bull age

This study demonstrated that age of bulls had highly significant effects (P<0.01) on all semen characteristics traits (Table 2). Our results are in agreement with previous studies (Boujenane & Boussaq 2013; Sitanggang 2018) in Holstein and Ongole crossbreed bulls which reported that age of bull affects all semen traits (ejaculate volume, sperm concentration, total sperm number and sperm motility). However, these results are not in agreement with previous report (Bhakat et al. 2011) which determined that the influence of age on sperm concentration has no significant effect in Sahiwal bulls.

Results for total sperm number followed those for ejaculate volume. The present findings are consistent with other studies (Snoj et al. 2013; Murphy et al. 2018). Ejaculate volume and total sperm number improved with the increasing age of bulls, but decreased again for the oldest ages (> 12 to 14.5 years). In different pattern, sperm concentration and sperm motility increased with age, but it began to decline after 9.5 years of age. The increase of ejaculate volume, total sperm number, sperm concentration and motility in older bulls were due to physiological changes of body mass augmentation (Balić et al. 2012), and the testicular growth and maturity (Moura et al. 2011; Rajak et al. 2014). However, Snoj et al. (2013) reported that reproductive decline in bull sires starts at different ages in different breeds.

In the current study, the highest ejaculate volume was observed in bulls > 9.5 to 12 years of age. This is similar to the findings of Snoj et al. (2013), with optimum value for Holstein bulls over 84 months of age.

Effect of season of collection

The current study shows that season only affected sperm motility (P<0.01) (Table 2). Season did not affect ejaculate volume, sperm concentration and total sperm number. Similar to the results, season of collection is reported to show significant effect on sperm motility in Karan Fries bulls (Bhakat et al. 2014). This present study contradicts to other researchers (Boujenane & Boussaq 2013; Snoj et al. 2013) in Holstein bulls. As reported by Boujenane & Boussaq (2013), the season had effects on all semen traits (ejaculate volume, sperm concentration, total sperm number and sperm motility) at AI Centers located in Morocco. Meanwhile, Snoj et al. (2013) observed that season affected ejaculate volume and total sperm number in AI Centers located in Slovenia.

Ejaculate volume and sperm motility were the highest during rainy season (4.88 mL and 61%, respectively) and the lowest during dry season (4.82 mL and 59%, respectively). In this study, there were significant differences (P<0.01) in sperm motility, but insignificant differences in ejaculate volume between rainy (October to March) and dry (April to September) seasons. Moreover, sperm concentration and total sperm number were the greatest during dry season (994 million/mL and 4.76 billion, respectively) and the smallest during rainy season (987 million/mL and 4.75 billion, respectively), but without significant differences. Similarly, Murphy et al. (2018) reported that semen collected in summer (May, June, July) and autumn (August, September, October) was of greater semen characteristics in term of sperm concentration and total sperm number than winter (November, December, January) and spring (February, March, April). Meanwhile, Boujenane & Boussaq (2013) reported the highest semen characteristics (ejaculate volume, sperm concentration, total sperm number and sperm motility) during the winter (January to March) and spring (April to June) season, and the lowest value in summer (July to September) and autumn (October to December) period. No clear pattern could be found to explain the effect of season on semen characteristics, but seasonal effects may be due to various factors, such as temperature, humidity, photoperiod, feed composition and management (Boujenane & Boussaq 2013).

Effect of ejaculation frequency

In the present study, ejaculation frequency affected all studied semen traits (P<0.01) (Table 2), except for sperm motility. The same results were found by Fuerst-Waltl et al. (2006) where the influence of frequency of ejaculation on ejaculate volume, sperm concentration

Table 1. Least square means, standard error and coefficients of variation for ejaculate volume, sperm concentration, total sperm number and sperm motility of Bali bulls

Characteristics	Number of data	Mean	Standard error	Coefficient of variation (%)
Ejaculate volume (mL)	3,847	4.85	0.03	37.31
Sperm concentration ($\times 10^6$/mL)	3,847	990.95	5.36	33.54
Total sperm number ($\times 10^6$)	3,847	4,759.03	35.57	46.36
Sperm motility (%)	3,847	60.23	0.27	27.67

Table 2. Least square means and standard error (SE) for semen characteristics of Bali bulls

Non-genetic factors	Number of data	Ejaculate volume (mL)	Sperm concentration ($\times 10^6$/mL)	Total sperm number ($\times 10^6$)	Sperm motility (%)
Age (years)					
> 2–4.5	1,334	4.24±0.04^{d}	982.92±7.73^{b}	4,180.45±51.58^{d}	59.83±0.46^{b}
> 4.5–7	1,501	4.92±0.05^{c}	1,022.72±9.59^{b}	4,968.27±60.45^{b}	60.95±0.42^{b}
> 7–9.5	487	4.77±0.08^{c}	1,093.12±13.03^{a}	5,206.33±100.76^{ab}	66.97±0.49^{a}
> 9.5–12	418	6.40±0.07^{a}	851.19±15.19^{c}	5,391.18±101.78^{a}	54.66±0.86^{c}
> 12–14.5	107	5.90±0.22^{b}	726.39±28.13^{d}	4,531.74±261.25^{c}	46.07±2.00^{d}
Season					
Rainy (October–March)	2,069	4.88±0.04	987.75±7.27	4,757.68±46.58	61.15±0.35^{a}
Dry (April–September)	1,778	4.82±0.04	994.66±7.94	4,760.59±54.67	59.15±0.41^{b}
Frequency of ejaculation					
First ejaculate	3,507	5.01±0.03^{a}	1,012.75±5.46^{a}	4,989.27±35.96^{a}	60.30±0.28
Second ejaculate	340	3.18±0.09^{b}	766.11±18.53^{b}	2,384.13±78.94^{b}	59.42±0.93
Collection intervals					
3 days	1,950	4.74±0.04^{b}	964.24±7.21^{b}	4,521.45±47.28^{b}	59.89±0.38
4 days	1,897	4.97±0.04^{a}	1,018.41±7.91^{a}	5,003.24±52.74^{a}	60.57±0.38

Means in the same column with different superscript highly significant (P<0.01)

Table 3. Repeatability estimations for semen characteristics of Bali bulls

Characteristics	Number of data	σ_s^2	σ_e^2	Repeatability
Ejaculate volume	3,847	1.48	1.94	0.43
Sperm concentration	3,847	40,207.93	72,764.36	0.35
Total sperm number	3,847	1,607,045.10	3,278,349.48	0.32
Sperm motility	3,847	89.38	197.67	0.31

σ_s^2, the sire variance; σ_e^2, the error variance

and total sperm number in Simmental bulls was observed. On the contrary, Bhakat et al. (2011) reported that frequency of ejaculation had non-significant effect on ejaculate volume in Sahiwal bulls. Whereas Murphy et al. (2018) and Sitanggang (2018) found there was ejaculation frequency on all studied semen characteristics of ejaculate volume, sperm concentration, total sperm number and sperm motility in Holstein and Ongole crossbreed bulls.

In general, the highest values for semen characteristics were found in the first ejaculates. First ejaculates were about 50%, 30% and 100% greater than second ejaculates for volume, sperm concentration and total sperm number, respectively. In accordance with the present findings, many other researchers (Karoui et al. 2011; Boujenane & Boussaq 2013; Murphy et al. 2018) reported that first ejaculates were superior in semen characteristics (ejaculate volume, sperm concentration and total sperm number).

Effect of collection interval

Table 2 shows that collection intervals affected ejaculate volume, sperm concentration and total sperm number ($P<0.01$). There was no significant effect of collection intervals on sperm motility. This is consistent with the findings of Fuerst-Waltl et al. (2006) who found no significant influence of collection intervals on sperm motility in Simmental bulls. This result is different from that described by Boujenane & Boussaq (2013) in Holstein bulls. They reported that there was significant effect of collection intervals on all semen traits (ejaculate volume, sperm concentration, total sperm number and sperm motility). Meanwhile, Karoui et al. (2011) in Holstein bulls reported that collection intervals had significant effect on ejaculate volume and total sperm number.

Collection intervals of 4 days exceed intervals of 3 days for semen characteristics. The differences were about 0.23 mL, 50 million/mL, 480 million and 0.68% for ejaculate volume, sperm concentration, total sperm number and sperm motility, respectively. According to the results for collection intervals, these indicated that Bali bulls produce optimum semen characteristics in intervals of 4 days. This study agrees closely to estimations of several studies (Fuerst-Waltl et al. 2006; Boujenane & Boussaq 2013). In Holstein bulls, Boujenane & Boussaq (2013) observed intervals of 4 days greater than intervals of 3 days for sperm concentration and total sperm number. Whereas, Fuerst-Waltl et al. (2006) found maximum intervals of 4-6 days for sperm concentration in Simmental bulls.

Genetic effect

With regard to the genetic effect, repeatability estimation of ejaculate volume, sperm concentration, total sperm number and sperm motility were 0.43, 0.35, 0.32 and 0.31, respectively (Table 3). These moderate to high repeatability estimations in recent study indicated that the selection of Bali bulls based on early performance records is reliable.

The repeatability of ejaculate volume in Bali bulls is higher than the estimations in Norwegian Red bulls at 0.29 (Olsen et al. 2020), and also higher than in Holstein bulls (Karoui et al. 2011; Boujenane & Boussaq 2013) at 0.31 and 0.411, respectively. Nevertheless, higher estimations were reported by Atagi et al. (2017) and Burren et al. (2019) which were at 0.467 and 0.63 in Japanese Black and Simmental bulls, respectively.

For sperm concentration, repeatability in the present study is greater compared to previous reports (Karoui et al. 2011; Boujenane & Boussaq 2013) in Holstein bulls which were at 0.30 and 0.175, respectively. However, it is smaller than Simmental bulls at 0.50 (Burren et al. 2019). A higher repeatability than the present estimation was also reported for Norwegian Red bulls at 0.43 (Olsen et al. 2020).

Repeatability estimations for total sperm number in Bali bulls seem to be higher than in Holstein and Ongole crossbreed bulls (Karoui et al. 2011; Sitanggang 2018) at 0.27 and 0.28. However, repeatability value in this study is lower than shown by another research (Atagi et al. 2017) was at 0.425 in Japanese Black bulls.

The repeatability value of sperm motility in this study is higher than Japanese Black and Norwegian Red bulls at 0.265 and 0.21 (Atagi et al. 2017; Olsen et al. 2020), however was lower than those shown by some studies of Karoui et al. (2011) and Burren et al. (2019) in Holstein and Simmental bulls at 0.35 and 0.52, respectively.

The difference in repeatability estimations by other studies might be due to the model, measurement and size of data used in the analysis (Lee et al. 2015).

CONCLUSION

Non-genetic factors of bull age, frequency of ejaculation and interval collection had a large effect on semen characteristics in Bali cattle. The bulls of >7 to 12 years of age were able to produce the highest semen characteristics. In general, the first ejaculate was the best as compared to the second ejaculate for semen characteristics. Semen characteristic of interval collection of 4 days was better than interval of 3 days. With regard to the genetic effect, repeatability estimated for semen characteristics of Bali bulls was in

moderate to high (0.31–0.43). The finding could help the AI Centre to optimize semen characteristics.

ACKNOWLEDGEMENT

The authors are grateful to the Singosari National Artificial Insemination Centre of Indonesia for providing the required data.

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Improving the Effects of Omega-3 Fatty Acid on the *In Vitro* Maturation of Oocytes

Ghorbani Vahed M[1,2], Khanbabaee R[3*], Shariati M[4], Edalatmanesh MA[2]

[1]*Department of Biology, Fars Science and Research Branch, Islamic Azad University, Fars, Iran*
[2]*Department of Biology, Shiraz Branch, Islamic Azad University, Shiraz, Iran*
[3]*Department of Biology, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran*
[4]*Department of Biology, Kazerun Branch, Islamic Azad University, Kazerun, Iran*
**E-mail: khanbabaeeramazan@gmail.com*

(received 27-08-2020; revised 28-10-2020; accepted 10-11-2020)

ABSTRAK

Ghorbani Vahed M, Khanbabaee R, Shariati M, Edalatmanesh MA. 2020. Peningkatan pengaruh asam lemak omega-3 dalam pematangan oosit secara *in vitro*. JITV 25(4): 153-161. DOI:http://dx.doi.org/10.14334/jitv.v25i4.2555

Penelitian ini dilakukan untuk mengetahui pengaruh omega-3 terhadap pematangan oosit secara *in vitro* dan tingkat ekspresi tribbles (gen *TRIB1*, *TRIB2* dan *TRIB3*) pada sel kumulus. Mencit NMRI berumur delapan-sepuluh minggu disuperovulasi menggunakan 7,5 IU serum gonadotropin (PMSG, Intraperitoneal) dari kuda bunting yang kemudian disembelih setelah 44 jam dan ovariumnya diangkat. Oosit digunakan untuk pematangan *in vitro* dan kompleks kumulus-oosit (COC) dilepaskan. Sel kumulus dan oosit dimasukkan ke dalam kontrol, dengan perlakuan etanol dan kelompok yang diberi 10 dan 100 µg/ml omega-3. Sel disiapkan guna melihat tahap pematangan untuk mengevaluasi tingkat ekspresi gen. Data dianalisis secara statistik. Mengekspos oosit dengan omega-3 dosis rendah (10 µg/ml) dan dosis tinggi (100 µg/ml) mengakibatkan penurunan laju oosit stadium GV, penurunan MI-oosit dan peningkatan MII-oosit. Peningkatan kematangan COC juga terdeteksi sebagai respon terhadap omega-3 dosis tinggi (100 µg/ml). Paparan sel kumulus terhadap omega-3 (10 dan 100 µg/ml) menginduksi *TRIB2* dan menghambat tingkat ekspresi gen *TRIB3*; namun, tingkat ekspresi gen *TRIB1* meningkat dan menurun sebagai respon terhadap konsentrasi omega-3 yang rendah (10 µg/ml) dan tinggi (100 µg/ml). Penambahan omega-3 ke dalam lingkungan oosit atau sel kumulus mempengaruhi pematangan oosit dan sel kumulus, yang diikuti oleh ekspresi diferensial dari gen *TRIB*, menunjukkan peran metabolisme asam lemak dalam diferensiasi dan pematangan sel kumulus.

Kata Kunci: Sel Kumulus, Pematangan, Omega-3, Gen *TRIB*

ABSTRACT

Ghorbani Vahed M, Khanbabaee R, Shariati M, Edalatmanesh MA. 2020. Improving the effects of omega-3 fatty acid on the *in vitro* oocyte maturation. JITV 25(4): 153-161. DOI:http://dx.doi.org/10.14334/jitv.v25i4.2555

This research was conducted in order to determine the effects of omega-3 on oocyte in vitro maturation and the level of expression of tribbles (*TRIB1*, *TRIB2* and *TRIB3* genes) in cumulus cells. Eight-ten weeks old NMRI mice were super-ovulated using 7.5 IU pregnant mare's serum gonadotropin (PMSG, Intraperitoneal) and they were killed after 44 hours and their ovaries were removed. The oocytes were used for *in vitro* maturation and the cumulus-oocyte complexes (COCs) were released. Cumulus cells and oocytes were assigned into control, ethanol-treated and groups exposed to 10 and 100 µg/ml of omega-3. The cells were prepared to assess the maturation stage in order to evaluate the gene expression level. The data were statistically analyzed. Exposing oocytes to low dose (10 µg/ml) and high dose (100 µg/ml) of omega-3 resulted in a reduced rate of GV-stage oocytes, decreased MI-oocytes and increased MII-oocytes. The enhanced maturity of COCs was also detected in response to a high dose of omega-3 (100 µg/ml). Exposure of cumulus cells to omega-3 (10 and 100 µg/ml) induced *TRIB2* and inhibited *TRIB3* gene expression level; however, *TRIB1* gene expression level increased and decreased in response to low (10 µg/ml) and high (100 µg/ml) concentrations of omega-3, respectively. The addition of omega-3 to the environment of oocytes or cumulus cells affected the maturation of oocytes and cumulus cells, which was followed by the differential expression of *TRIB* genes, suggesting that there was a role of fatty acid metabolism in the differentiation and maturation of cumulus cells.

Key Words: Cumulus Cells, Maturation, Omega-3, Oocyte, *TRIB* Genes

INTRODUCTION

There are many clinical and experimental data demonstrating the health benefits of omega-3 fatty acids in healthy individuals as well as patients with reproductive failures (Jeromson et al. 2015; Cao et al. 2015). *In vivo* and *in vitro* studies represent that many aspects of reproductive system, including oogenesis and spermatogenesis, are influenced by omega-3 fatty acids metabolism (Nehra et al. 2012; Gulliver et al. 2012;

Meher et al. 2013). Omega-3 fatty acids have also a significant role in oocyte maturation as well as oocyte quality (Chiu et al. 2018; Ortiz et al. 2014). *In vivo* experiments have shown that intake of foods and supplements containing omega-3 acids contributes to granulosa cells and oocytes growth and development (Wonnacott et al. 2020). Since ovarian compartments are composed of fatty acids, dietary n-3 fatty acids can modify the ovarian compartments, resulting in improved ovarian structure and function (Zachut et al. 2010). Polyunsaturated fatty acids are major components of the granulosa cells surrounding oocytes and contribute to oocytes maturation and therefore play significant role in female fertility (Khalil et al. 2013; Shaaker et al. 2012). In this condition, *in vitro* studies have indicated that during oocyte maturation, unsaturated lipids are incorporated into the oocyte cytoplasm and influence cellular metabolism and oocyte growth and development (Carro et al. 2013).

Fatty acids involved in maturation of oocytes can regulate expression level of genes associated with oocyte maturation (Veshkini et al. 2016; Virant-Klun et al. 2013). Recently, certain gene expression profiles have been studied and the findings indicate that oocyte maturation and follicular growth involve the expression of a number of genes including *Tribbles* (*TRIB*) genes (Hernández-Montiel et al. 2019; Lussier et al. 2017; Brisard et al. 2014). However, *TRIB* genes have been less studied and the modulation of their expression during oocyte maturation is somehow unclear. The association of Tribbles, *TRIB1*, *TRIB2* and *TRIB3*, with fatty acid metabolism has also been demonstrated in several studies. The findings indicate that *Tribbles* regulate cell proliferation in many tissues partly due to their impact on fatty acid metabolism (Lohan & Keeshan 2013). *TRIB1* has been reported to promote lipid metabolism in human tissues (Legault et al. 2018). However, *TRIB3* prevents fat accumulation in adipocytes (Örd et al. 2015; Lirangi et al. 2012). The association of *in vivo* oocyte maturation and *TRIB1* gene expression level has been reported (Brisard et al. 2014). *TRIB2* gene expression level is also changed in granulosa and cumulus cells during oocyte development (Lussier et al. 2017; Assidi et al. 2010).

Altogether, these observations hypothesized and somehow revealed that omega-3 fatty acids may influence *in vitro* oocyte maturation as a microenvironment. Fatty acids also can alter expression level of *Tribbles* in follicular cells. However, there are not sufficient findings clearly showing the association of omega-3 fatty acids with *Tribbles* expression level alteration during oocyte maturation. In this condition, the purpose of this research was to determine the effects of omega-3 on oocyte maturation *in vitro* and expression level of *Tribbles* (*TRIB1*, *TRIB2* and *TRIB3* genes) in cumulus cells surrounding oocytes.

MATERIALS AND METHODS

Chemicals and reagents

Omega-3-acid ethyl esters capsules were purchased from Pronova Biopharma Norway Pharmacy Company. Ethanol (96%) (as solvent) was added to content of each capsule to prepare the desired solution.

Collection of oocytes

This experimental study was conducted on 100 female NMRI mice, aged 8-10 weeks that were maintained on a 12-12 h light-dark schedule with *ad libitum* access to food and water. According to previous studies (Sirard 2011; Nikseresht 2015) animals were super-ovulated by an intraperitoneal injection of 7.5 IU pregnant mare serum gonadotrophin [PMSG Intervet, UK]. Ether was used to anesthetize the animals and the ovaries were removed into TCM-199 [Sigma] supplemented with 10% fetal bovine serum (FBS). The ovaries were punctured and cumulus-oocyte complexes (COCs) were released under a stereomicroscope. Oocytes were used for *in vitro* maturation. The study protocol was approved by the ethics committee of Islamic Azad University, Shiraz, Iran (Ethical no: IR.IAU.SHIRAZ.16330641322009).

In vitro maturation (IVM)

The oocytes and cumulus cells were cultured in α-MEN culture medium containing 5% FBS, 0.1 IU/mL, LH rh FSH, 7.5 IU/mL and 1% Penicillin-Streptomycin. The cells were assigned into 4 groups: Control (no treatment), Ethanol treated (exposed to ethanol 96%), Experimental 1 and Experimental 2 (exposed to 10 and 100 μg/ml of omega-3 fatty acid, respectively). The cells were incubated for 24h at 38.58˚C under 5% CO_2 in humidified air and prepared for determination of maturation stage and evaluation of gene expression level in cumulus cells.

Assessment of oocyte maturation rate

Oocytes were stripped from cumulus cells by repetitive aspiration-ejection using a Gilson pipette and then immediately fixed in 4% paraformaldehyde phosphate buffered saline (PBS) solution at room temperature for 30 minutes. Hoechst33342 (0.1% in PBS) was used for incubating fixed oocytes for 10 minutes and mounted on a glass slide in Mowiol solution. The maturation quality of oocytes was determined according to previous studies (Brisard et al. 2014). In the case of seeing the telophase-I or metaphase-II stage, the oocytes were considered mature. As a percentage of mature oocyte in a total

Table 1. Specific primers for *TRIB1*, *TRIB2* and *TRIB3* genes

Genes	Parameters		
	Sequence (5'->3')	Annealing (°C)	Product Size (bp)
Trib1-F	CCTCGAATATGGCAGCATTT	60	101
Trib1-R	CGAGTCTCCTCACCCTTGTC		
Trib2-F	TTGGAACAGACCAACCACCT	60	98
Trib2-R	TTTAGCACCCAGGTTTCAGG		
Trib3-F	GGAACCTTCAGAGCGACTTG	60	101
Trib3-R	TCTCCCTTCGGTCAGACTGT		
Rplp0-F	TGCCACACTCCATCATCAAT	60	97
Rplp0-R	AGGAAGGCCTTGACCTTTTC		

F: Forward, R: Reverse

number of live oocytes, the maturation rate was calculated.

Evaluation of *Tribble* expression level

Cumulus cells of COCs at maturation state were seeded in dishes. Twentyfour hours after seeding, the cells were incubated with the ethanol and omega-3 (10 and 100 µg/ml) for 12h. Cells were then centrifuged and washed with ice-cold PBS. Total RNA was extracted using a RNeasy midi kit [Roche, 1 828 665, Germany] and reverse transcribed into cDNA using a Transcriptor First Strand cDNA synthesis kit [Roche,04 379 012 001, Germany]. Quantitative Real-Time PCR (LightCycler-FastStart DNA master SYBR Green I Kit [ABI, 4369016, American] and Light Cycler apparatus [Roche Diagnostics]) was carried out to evaluate the expression level of *TRIB1*, *TRIB2* and *TRIB3* genes using the specific primers (Table 1). RT-PCR reaction was conducted during a program at a temperature of 95° C for 15 minutes and was subjected to 40 cycles compose of three-steps program at 95°C for 10 second, at 60°C for 30 second and at 72°C for 30 second. *Rplp0* gene (as reference gene) was used to normalize the relative expression for interested genes calculated by $2^{-\Delta\Delta CT}$ method. An agarose gel electrophoresis was used to confirm the expected PCR products.

Data analysis

Results are expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA method) followed by post hoc Tukey's multiple comparisons test in SPSS 20 software. Differences were considered significant at the $P<0.05$ level.

RESULTS AND DISCUSSION

Result

Maturation status of oocytes

Oocytes were examined in control, ethanol treated and experimental groups at 24h of IVM. Table 2 indicates the status of oocytes in control and ethanol treated groups and groups exposed to 10 and 100 µg/ml of omega-3 at 24 hours of IVM. Exposure of oocytes to 10 µg/ml of omega-3 resulted in lower degenerated oocytes and to 100 µg/ml of omega-3 led to higher degenerated oocytes compared with control group. The rate of GV-stage oocytes was significantly lower in groups exposed to 10 and 100 µg/ml of omega-3 than control group. Adding omega-3 (10 and 100 µg/ml) to live oocytes environment resulted in significant decrease in oocytes arrested at MI stage and significant increase in the rate of oocytes arrested at MII stage compared to control group.

Maturation status in COCs

Complete maturation was also examined in pre-ovulatory COCs in control, ethanol treated and experimental groups 34–38h after triggering ovulation.

COCs were graded by observational evaluation of morphological features such as the thickness and compactness of the cumulus cells and ooplasm homogeneity. According to previous studies, based on light microscopy, COCs surrounded by several layers of cumulus cells and with evenly granulated ooplasm were considered to have higher developmental competence *in vitro* (complete maturation) than oocytes with irregularly granulated ooplasm and fewer cumulus layers (Gumus et al. 2010).

Reduced maturity was observed in COCs exposed to 10 µg/ml of omega-3; however, exposure of COCs to 100 µg/ml of omega-3 led to an increased maturity rate (Figure 1). The rate of COCs at MII stage risen significantly in the group exposed to 10 µg/ml of omega-3. However, adding 100 µg/ml of omega-3 to COCs environment resulted in decreased COCs at MII stage. GV/MI rate in COCs was significantly lower in the group exposed to 10 µg/ml of omega-3 and was considerably higher in the group exposed to 100 µg/ml of omega-3 compared with control group (Figure 2).

TRIB genes expression level in cumulus cells

TRIB1, *TRIB2* and *TRIB3* genes expression level was evaluated in cumulus cells of COCs at complete maturation stage (Table 3). Exposure of COCs to 10 µg/ml of omega-3 resulted in reduced expression level of *TRIB1* gene and exposure to 100 µg/ml of omega-3 led to significant increase in *TRIB1* gene expression level. *TRIB2* gene expression level significantly increased in groups exposed to 10 and 100 µg/ml of omega-3, however, the expression level was higher in the group exposed to 10 µg/ml of omega-3 than the group exposed to 100 µg/ml of omega-3. *TRIB3* gene expression level significantly decreased in groups exposed to 10 and 100 µg/ml of omega-3. We did not observe significant difference between ethanol treated and control groups in our experiments, indicating that ethanol (as solvent) did not have significant impact on the research data

Discussion

Our findings indicated that exposure of live oocytes to low (10 µg/ml) and high (100 µg/ml) dose of omega-3-acid ethyl ester resulted in reduced rate of GV-stage oocytes, decreased MI-oocytes and increased MII-oocytes, demonstrating the stimulatory effects of omega-3 on maturation of live oocytes *in vitro*. In addition, our results have revealed that adding the low (10 µg/ml) and high (100 µg/ml) dose of omega-3 to COCs environment increased and decreased maturity of COCs, respectively.

In line with our findings numerous studies have reported that unsaturated fatty acids and omega-3 derivatives play significant role in maturation of oocytes and granulosa cells in various species. Indeed, long-chain poly unsaturated fatty acids such as omega-3 and omega-6 are integral component of the membrane lipid bilayer in many types of cells, including reproductive system cells (Gulliver et al. 2012), by which may influence cell function and development. Studies have shown that the dietary supplementation of omega-3 can improve reproductive system function in different species (Kirkup et al. 2010; Safdar et al. 2017). A large body of experimental research has also demonstrated that omega-3 improves female reproductive system function by affecting on female hormones precursors and the genes associated with oogenesis (Gulliver et al. 2012; Cheng et al. 2013; Dirandeh et al. 2015). Dietary n-3 fatty acids have been reported to influence the follicular status and to increase the cleavage rate of oocytes (Zachut et al. 2010). *In vivo* investigations on oocyte and embryo development have revealed that regular intake of unsaturated fatty acids has improving effects on oocyte maturation and embryo development (Fayezi et al. 2018). Fatty acids can also modulate granulosa cell proliferation and steroidogenesis *in vitro* (Maillard et al. 2018), by which may influence cumulus-oocyte complexes (COCs) maturation. The data obtained in recent studies confirm the significant association of unsaturated fatty acids with oocyte maturation and implantation (Mirabi et al. 2017; Oseikria et al. 2016; Mahla et al. 2017).

The cytotoxic and genotoxic effects of unsaturated fatty acids on oocytes and cumulus cells during IVM have been investigated (Nikoloff et al. 2017). The previous studies have suggested that the effect of fatty acids on *in vitro* systems depends in part on the concentration of unsaturated fatty acids and the cell type used in the study (Meng et al. 2013; Zajdel et al. 2013). The use of low concentrations of unsaturated fatty acids such as eicosapentaenoic acid can improve oocyte quality and cumulus expansion, while its high concentrations can induce cytotoxic and genotoxic effects. In the present study, the use of low dose of omega-3 decreased degenerative oocytes and the high dose of omega-3 increased degenerated oocytes, which is consistent with the previous studies (Nikoloff et al. 2017). The relationship between oocytes and cumulus cells is established through a gap junction so that any change made in these cells reduces the quality of oocytes (Zhou et al. 2016). It has been observed that high concentrations of unsaturated fatty acids such as eicosapentaenoic acid can induce apoptosis in cumulus cells. Also, the cytotoxic effects of unsaturated fatty acids at high concentrations can be associated with decreased metabolic activity and decreased mitochondrial activity. The increase in degenerative oocytes in this study can be explained by this possible mechanism that unsaturated fatty acids at high concentrations may be suitable targets for the formation

Table 2. Effect of ethanol and omega-3 on the *in vitro* maturation of oocytes in mice

Parameters	Groups			
	Control	Ethanol-treated	Exp1 (10 µg/ml)	Exp2 (100 µg/ml)
Degenerated (%)	7.6	8.6 ^{ns}	5.8$^{1, *}$	10.8$^{1, **; 2, *}$
GV (%)	13	11.8 ^{ns}	5$^{1, ***}$	8.1$^{1, **; 2, *}$
Metaphase I (%)	15.2	14 ^{ns}	7.9$^{1, ***}$	3.6$^{1, ***; 2, *}$
Metaphase II (%)	64.2	65.6 ^{ns}	81.3$^{1, ***}$	77.5$^{1, ***; 2, *}$
Number of oocytes	150	150	150	150

GV: arrested at germinal vesicle state (nucleus of oocyte is arrested in prophase of meiosis I)
[1] and [2] indicate significant difference compared with control and group exposed to 10 µg/ml of omega-3 at 24h of IVM, respectively; ns: indicates non-significant difference. *: P≤0.05, **: P≤0.01, ***: P≤0.001

Table 3. Effect of ethanol and omega-3 on the relative gene expression level (RQ) of *TRIB1*, *TRIB2* and *TRIB3* in cumulus cells

Genes	Groups			
	Control	Ethanol-treated	Exp1 (10 µg/ml)	Exp2 (100 µg/ml)
TRIB1	1.03±0.02	1.14±0.08 ^{ns}	4.28±0.22$^{1, **}$	0.38±0.03$^{1, ***; 2, ***}$
TRIB2	0.38±0.02	0.44±0.01 ^{ns}	4.85±0.24$^{1, **}$	2.36±0.04$^{1, **; 2, **}$
TRIB3	2.30±0.21	1.94±0.41^{ns}	1.55±0.32$^{1, **}$	0.76±0.06$^{1, **; 2, **}$

Data represents relative gene expression (Target/Rplp0) mean ± SEM of three experiments (n=3). [1] and [2] indicate significant difference compared with control and group exposed to 10 µg/ml of omega-3, respectively; ns: indicates non-significant difference, **: P≤0.01, ***: P≤0.001

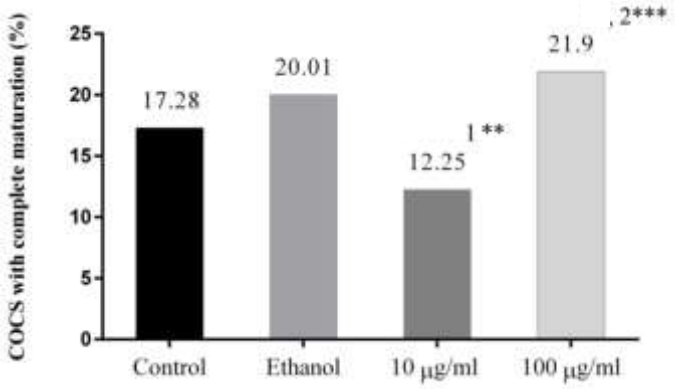

Figure 1. Complete maturation in control, ethanol treated COCs and COCs exposed to 10 and 100 µg/ml of omega-3 at GV/MI stage. [1] and [2] indicate significant difference compared with control group and group exposed to 10 µg/ml of omega-3, respectively (*: P≤0.05, **: P≤0.01, ***: P≤0.001).

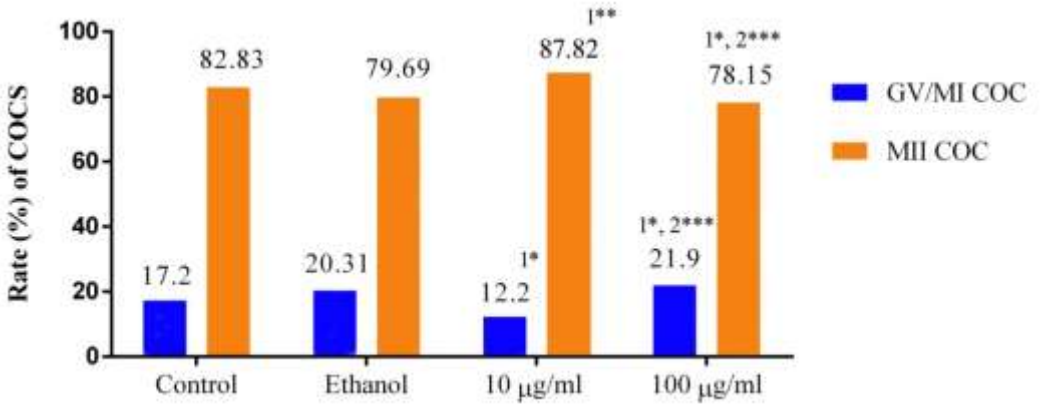

Figure 2. The rate (%) of COCs at GV/MI and MII stages in control and ethanol treated groups and groups exposed to 10 and 100 µg/ml of omega-3. [1] and [2] indicate significant difference compared with control group and group exposed to 10 µg/ml of omega-3, respectively (*: P≤0.05, **: P≤0.01, ***: P≤0.001).

of free radicals. The formation of free radicals after long-chain peroxidation of unsaturated fatty acids can be the main cause of damage to DNA and proteins, resulting in reduced quality and maturation of oocytes (Nikoloff et al. 2017). Therefore, based on the findings of this study, it seems that the high dose of omega-3 has destructive effects on oocyte maturation.

In the present study we found that exposure of COCs to low dose of omega-3 resulted in increased maturation and to high dose of omega-3 led to decreased maturity of COCs. The findings of a recent study have shown that the maturation parameters of COCs were affected by exposure of COCs to different eicosapentaenoic acid concentrations in the IVM medium and higher and lower concentrations of eicosapentaenoic acid have different impacts on COCs maturation (Nikoloff et al. 2017). In contrast to our findings, there are research showing that fatty acids may retard growth and development in oocytes. It has been reported that the exposure of oocytes to an environment high in omega-3 fatty acids led to decreased developmental ability of the blastocyst stage (Dunning et al. 2010). Adding alpha-linolenic fatty acids to oocyte environment also has been shown to increase reactive oxygen species levels, which mediate, at least in part, the inhibitory effect on oocyte maturation (Marei et al. 2012). Higher levels of saturated fatty acids, especially palmitic and stearic acids, were observed in some metabolic contexts to have harmful effects on oocyte maturation (Mirabi et al. 2017).

Results of this study indicated that adding omega-3 (10 and 100 µg/ml) to COCs environment induced *TRIB2* and inhibited *TRIB3* gene expression in cumulus cells. However, *TRIB1* gene expression level increased and decreased in response to low (10 µg/ml) and high (100 µg/ml) concentrations of omega-3, respectively. In line with this findings a research carried out to evaluate *TRIB* genes expression level in pre-ovulatory follicles indicated that *TRIB1*, *TRIB2* and *TRIB3* are expressed in different patterns in cumulus cells surrounding the oocytes from pre-ovulatory follicles (Brisard et al. 2014), and therefore, it is expectable that adding different doses of omega-3 fatty acids to cumulus cells has different effects on *TRIB* genes expression level. Previous studies also have revealed a significant relationship between *TRIB* genes expression level and fatty acid metabolism in cumulus cells during oocyte maturation. It has been shown that *TRIB* genes are involved in the cell-cycle progression during cell division (Dugast et al. 2012), which is accompanied by an increase in cellular metabolism rate including fatty acids metabolism. Indeed, maturation process is associated with significant change in COCs lipid metabolism which at least in part is regulated by *TRIB* genes expression (Bauer et al. 2015). A link between *TRIB1* gene expression level and lipid metabolism has been reported in recent studies (Wang et al. 2015). The findings show that the expression level of *TRIB3* has significant impact on carbohydrate metabolism (Zhang et al. 2016), which in turn, may influence lipid metabolism as well. Although the findings of our research indicated that adding omega-3 to COCs environment upregulates *TRIB2* and downregulates *TRIB3* in cumulus cells, it has been previously shown that *TRIB3* was up-regulated and *TRIB2* was down-regulated during the preovulatory period in cumulus cells (Lussier et al. 2017).

The studies indicate that all the three *TRIB* genes are regulated differently at different times in response to the inhibition of fatty acid peroxidation. This confirms that the three *TRIB* genes are involved in fatty acids metabolism and cumulus cells proliferation and play a key role in the resumption of meiosis and oocyte maturation (Brisard et al. 2014).

In this study, increased expression of *TRIB1* and *TRIB2* and decreased expression of *TRIB3* is associated with increased maturation of the COCs at low dose (10 µg/ml) of omega-3, however, at the maximum dose (100 µg/ml) of omega-3, the expression level of the TRIB genes were significantly reduced compared to the low dose (10 µg/ml) group of omega-3. Based on these findings, omega-3 appears to have a dose-dependent effect on gene expression and maturation of the COCs. Because a few studies are available on the effects of *TRIB* genes expression on the COCs, and the effects of omega-3 on different genes are variable, reducing the expression of *TRIB1* at the maximum dose (100 µg/ml) of omega-3 could be due to the cytotoxic effects of omega-3 at high doses in the form of impaired metabolic activity and the formation of free radicals due to peroxidation of fatty acids (Wu et al. 2015). Decrease in *TRIB1* gene expression at the high dose (100 µg/ml) of omega-3 can also be attributed to an increase in COCs at the GV/MI stage, so that the decrease of oocytes at GV/MI stage is associated with an increase in *TRIB1* expression and its increase is associated with decreased *TRIB1* expression in this study.

Our study only investigated the relationship between omega-3 and expression level of *Tribbles* in cumulus cells *in vitro*; however, to clarify the exact mechanism of omega-3 action on oocytes and cumulus cells, further research are required to reveal the effects of omega-3 on expression level of genes and proteins associated with oocyte and cumulus cells maturation *in vitro* and *in vivo*.

CONCLUSION

In conclusion, the data obtained in the present study suggest that adding omega-3-acid ethyl ester to environment of oocyte and cumulus cells promotes maturation of live oocyte and cumulus cells *in vitro* which is accompanied by increasing of *TRIB2* and decreasing of *TRIB3* gene expression. It is suggested that supplementation of diet with omega-3 may improve the oocyte development in patients suffering failure in oocyte maturation.

ACKNOWLEDGEMENT

This paper has been extracted from the PhD dissertations in developmental biology approved and supported by Shiraz Branch, Islamic Azad University, Shiraz, Iran. We appreciate all who helped us to exert this research.

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Reproduction Status and Population Dynamic of Kuantan Cattle in the Kuantan Singingi Regency

Yendraliza[1], Rodiallah M[1], Astuti T[2], Elfawati[1]

[1]*Department of Animal Science, Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau*
Jl. H.R. Soebrantas KM 15 Simpang Baru, Pekanbaru, Riau, Indonesia 28293
[2]*Departement of Animal Science, Faculty of Agriculture, University Mahaputra Muhammad Yamin*
Jl. Jendral Sudirman No.6, Solok, West Sumatera. Indonesia.
E-mail: yendraliza@uin-suska.ac.id

(received 29-07-2020; revised 26-10-2020; accepted 11-11-2020)

ABSTRAK

Yendraliza, Rodiallah M, Astuti T, Elfawati. 2020. Status Reproduksi dan dynamic populasi sapi Kuantan di Kabupaten Kuantan Singingi. JITV 25(4): 162-172. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2541

Efisiensi reproduksi, struktur populasi, dinamika populasi, natural increase, estimasi output merupakan salah satu tolak ukur menentukan ternak dan wilayah tersebut dapat dijadikan sumber bibit. Tujuan penelitian ini adalah untuk menentukan efisiensi reproduksi, dinamika populasi, natural increase dan estimasi output populasi sapi Kuantan di Kabupaten Kuantan Singingi, Provinsi Riau, Indonesia. Penelitian menggunakan 311 ekor sapi Kuantan dan 99 orang peternak sapi Kuantan. Pengambilan sampel menggunakan purposive sampling dengan metode survey dan pengamatan langsung, data dianalisis secara deskriptif. Parameter yang diukur adalah efisiensi reproduksi, natural increase, estimasi output dan dinamika populasi sapi Kuantan. Hasil penelitian memperlihatkan bahwa efisiensi reproduksi sapi Kuantan adalah 1.04 %, natural increase 5.14%, imbangan jantan dan betina 1:5, nilai NRR jantan 50% dan NRR betina 100.56 %, total ternak yang keluar 18.69 % dan total ternak yang masuk 18.69%, nilai output 48.88 % dan estimasi dinamika populasi 2.85%. Kesimpulan, reproduksi sapi kuantan belum efisien dengan pertambahan alami sapi kuantan amat rendah dan jumlah ternak penganti sapi jantan dan betina belum terpenuhi. Disarankan untuk tidak mengeluarkan sapi Kuantan dalam 5 tahun kedepan untuk menjaga keseimbangan populasi.

Kata Kunci; Dinamika Populasi, Natural Increase, Output, Reproduksi

ABSTRACT

Yendraliza, Rodiallah M, Astuti T, Elfawati. 2020. Reproduction status and population dynamic of Kuantan cattle in the Kuantan Singingi Regency. JITV 25(4): 162-172. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2541

The purpose of this study was to determine reproductive efficiency, population dynamics, natural increase and estimated output of the Kuantan cattle in the Kuantan Singingi Regency, Province of Riau, Indonesia. A total of 311 Kuantan cattle and 99 Kuantan cattle farmers were used in this study through a survey study. Respondent samples were taken from seven districts. Data sampling using purposive sampling with survey methods. Data collection was carried out by interviewing farmers and observing and was analyzed descriptively. Parameters measured were reproductive efficiency, natural increase, estimated output and population dynamics of Kuantan cattle. Results showed that the reproductive efficiency of Kuantan cattle was 1.04%, natural increase 5.14%, the balance of male and female 1: 5, the value of male NRR 50% and female NRR 100.56%, total cattle out 18.69% and total incoming cattle 18.69%, output value 48.88% and estimated population dynamics 2.85%. In conclusion, Kuantan cattle reproduction has not been efficient with the natural increase of the Kuantan cattle was very low, and the replacement stock availability for male and female cattle has not been fulfilled. It is recommended not to release Kuantan cattle in the next 5 years to maintain population balance.

Key Words: Natural Increase, Output, Population Dynamic, Reproduction

INTRODUCTION

The Indonesian policies about meat self-sufficiency base on local resources start in 2000- 2010, but unsuccessful because of a lack of information about local cattle populations, geographical distribution, and genetic characteristics. The native cattle play a vital role in Indonesia's socioeconomic, conservation and breeding programs for Indonesian cattle breeds must be well designed based on their potential and genetic information (Agung et al. 2019). The Kuantan cow is one of Indonesia's local cows from Riau with a decree Ministry of Agriculture Indonesia as No. 1052/kpts/S.R.120/10/2014. Kuantan cattle are smaller in size than Aceh cows and Bali cows. The results of genetic distance analysis show that Kuantan and Pesisir cattle have close genetic values and are in the Bos indicus group (Hidayati et al. 2016). Kuansing Regency

is one of the original areas of Kuantan cattle. Kuantan cattle have the potential to be developed because they are raised by breeders in rural areas of the Kuantan and Indragiri rivers, easily adaptable to low feed nutrition and resistant to disease (Department of Animal Husbandry and Animal Health of Riau Province, 2019). The same data source states that the population of Kuantan cattle in the Kuansing Regency has not increased from 2014(1.179 heads) to 2018 (1.278 heads). Evaluation of population growth or population dynamics to maintain population balance is important (Rohyan et al. 2016). Estimated output data, natural increase, birth rates, and livestock mortality are used to determine livestock breeding patterns (Warwick et al. 1983). The age composition of the livestock population is needed to determine the need for replacement cattle in one year. The population dynamics will give clues on the population viability in the future (Wang et al. 2016). Breeding patterns are closely related to the appearance of livestock reproduction. Literature investigation, research that has been done on Kuantan cattle is sperm quality in Kuantan cattle (Jiyanto & Anwar, 2019). The Population dynamics of Pesisir cattle was carried out in (Putra et al. 2015), Madura cattle (Kutsiyah, 2017), Bali cattle (Budiarto et al. 2013), breed Ongole (Kusuma et al. 2017); (Rohyan et al. 2016) and Pasundan cattle (Said et al. 2017). Growth data, population dynamics, natural increase and reproductive efficiency of Kuantan cattle have not been done. This data is needed to determine the ability of livestock inbreeding and the ability of the region to be a source of breeders, substitute livestock and fattening. The purpose of this study was to determine reproductive efficiency, population dynamics, output, natural increase and mutation of Kuantan cattle in the Kuansing District.

MATERIALS AND METHODS

This research was conducted in Benai, Inuman, Pangean, Cirenti, Singingi Hilir, and Singingi Districts, Kuantan Singingi (Kuansing) Regency for six months, starting from June to November 2019 (Figure 1). The selection of research locations is based on the largest number of Kuantan cattle populations. The materials used were 99 farmers owning Kuantan cattle with 311 Kuantan-cows after parturition. Kuantan cattle are grazing long days and placed in the barns at night. The feed given was a grass field, lict salt as a feed additive, drinking water was given ad-libitum.

This research location and respondent were the determination of the purposive sampling method. They were selected based on the Kuantan cattle population. The respondent sample was Kuantan cattle farmers who had 5 years' experience in reproduction management. Data were collected by direct interviews with respondents using questionnaires and observation of livestock for body condition after parturition. The interviews were done about the identity of the farmer, the number of livestock ownership, livestock population, the first mating age, rearing limitation of age, weaning age, first mating after post-partum, calving interval, number of calves cows, mutations of livestock and the age of one year, and the method of mating.

Data analysis

The reproductive data were analyzed quantitatively by displaying the mean and standard deviation. The data number of livestock, population structure be used to calculate the natural increase, net replacement rate, and estimated output through the livestock breeding approach.

Population structure was calculated and analyzed according to Warwick et al. (1983), as follow:

$$\text{Number of adult cattle (\%)} = \frac{\text{Number of adult cattle}}{\text{Number of the adult population}} \text{x } 100\%$$

Calving rate based on female adult cattle or calf crop was calculated based on the number of female adults and number of population:

$$\text{Calving rate based on the number of female adults (\%)} = \frac{\text{Number of calves}}{\text{Number of female adults}} \text{x } 100\%$$

$$\text{Calving rate based on the number of population (\%)} = \frac{\text{Number of calves}}{\text{Number of population}} \text{x } 100\%$$

$$\text{Mortality(\%)} = \frac{\text{Number of death cattle/year}}{\text{Number of population/year}} \text{x } 100\%$$

Natural Increase

$$\text{calving rate base on population} - \text{mortality}$$

Net Replacement Rate (NRR) =

$$\frac{\text{number of replacement young cattle candidates}}{\text{need for replacement cattle}} x\ 100\%$$

If NRR <100%, then the need for substitute livestock is not fulfilled, conversely if NRR> 100%, the need for replacement livestock is fulfilled

Replacement stock necessary for male cattle (%) is obtained from the number of adult males (heads) divided by the rearing limitation age of male cattle (years). Replacement stock necessary for female cattle (%) is obtained from the number of adult females (heads) divided by the rearing limitation age of female cattle (years).

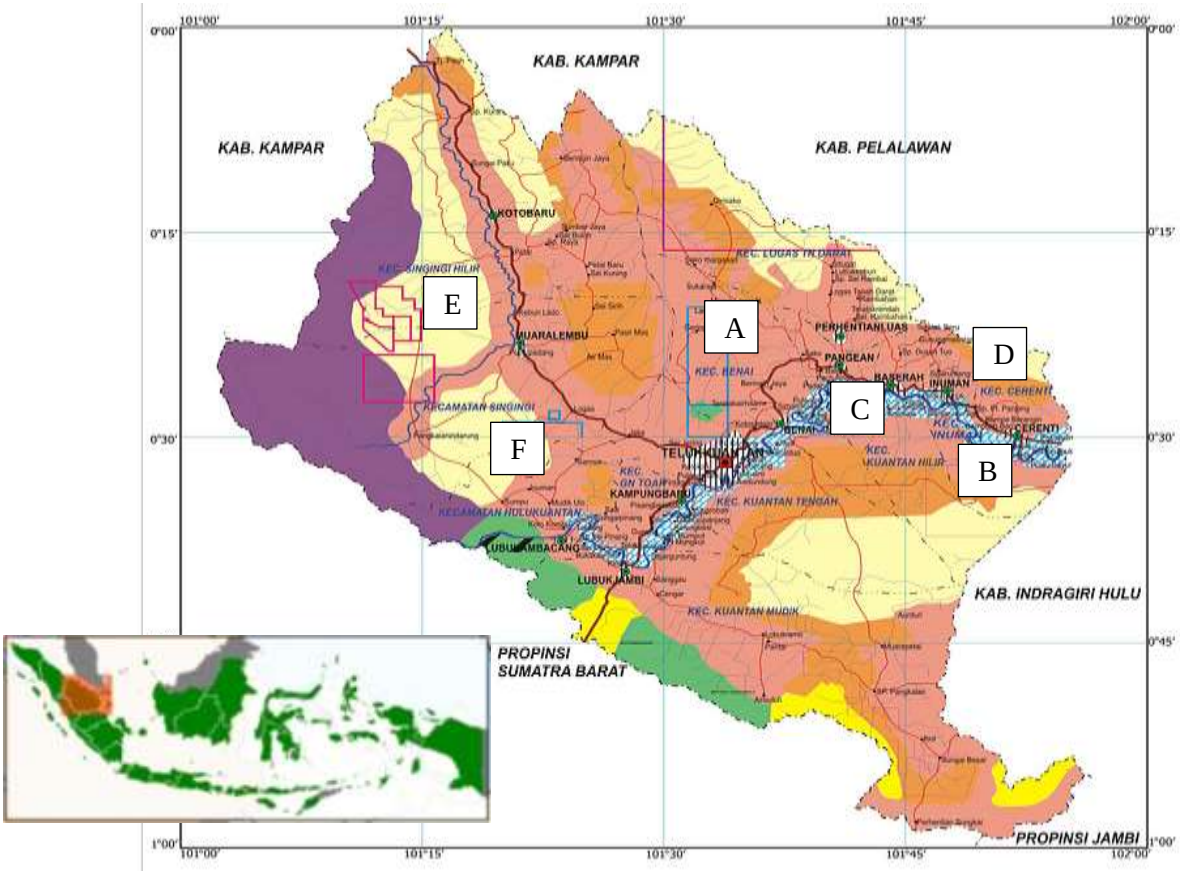

Figure 1. Location of the research site at six Districts at Kuantan Singingi Regency, Riau Province, Indonesia consisted of: A. Benai, B. Inuman, C. Pangean, D. Cirenti, E. Singingi Hilir and F. Singingi districts.

Population dynamics were estimated from the Kuantan cattle population data in Kuansing District over the past five years with time series analysis of the least-squares method with a linear polynomial equation.

The cattle output rate was calculated as the male cattle of replacement (%) + the female cattle of replacement (%) + the culling cattle of male + the culling cattle of the female.

Female cattle growth

$$= \frac{\text{number of female cattle calves (heads)}}{\text{total population (heads)}} X\ 100\%$$

Male cattle growth

$$= \frac{\text{number of male cattle calves (heads)}}{\text{total population (heads)}} X\ 100\%$$

The rest of the replacement for young male cattle was calculated as male growth (%) - the replacement stock necessary for male cattle (%). Where the rest of the replacement for young female cattle was calculated as female growth (%) - the replacement stock necessary for female cattle (%).

The culling male cattle were calculated as the rest of the replacement for young male cattle, where the culling female cattle was calculated as the rest of the replacement for young female cattle.

Reproduction Efficiency (RE) was measured according to Sumadi (2001) as:

$$(\text{calving interval x number of calf})/((\text{first} - \text{time calving} - \text{first time mating}) + (\text{calving interval} - \text{duration of pregnancy})) \text{ x } 100\%$$

RESULTS AND DISCUSSION

Characteristics of Kuantan cattle breeders

The average age of respondents is 36-49 years, with the greatest level of education being senior high school. have 10-15 years of experience raising livestock with 4-9 cattle per family. The main occupation of the respondents is raising livestock with 4-5 laborers per family carried out by family members themselves. The average age of respondents is still a productive age with a longer experience of raising livestock and a relatively

high level of education allows for more learning, accepting technological innovations towards better change, but the knowledge they gain is only from experience. So that management is carried out only from generation to generation. This can be seen from the management of feed, which is done only by providing field grass without concentrate. For breeding management, breeders have already carried out livestock germ selection (Figure 2). But not all farmers do recording, judging and replacement stock. Characteristics of respondents are an indicator to determine the ability of farmers to manage their livestock (Romjali 2018).

Kuansing land area

The area of pasture land in the Kuansing Regency is 1,831 Ha (Table 1). The capacity of pasture in the Kuansing Regency is 5.016,44 ST or equivalent to 5.016 adult cattle with a weight of 250 kg. The current population of Kuantan cattle is 1.278 (estimated data), so there is an opportunity to increase the population of 3.738,44 adult cattle. This opportunity becomes greater if the area of pasture is combined with other agricultural areas such as plantations, paddy fields, yards, and fields. This condition allows the Kuansing regency to become a place for developing Kuantan cattle.

Reproduction characteristics of Kuantan cattle

Characteristics of livestock reproduction are useful for knowing the reproductive ability of livestock to produce subsequent offspring. The mean age at first mating in male and female Kuantan cattle was 24.5 ± 3.03 months and 45.6 ± 1.93 months (Table 2). This value is greater than Pesisir cows (22.67 ± 2.53 months and 23.02 ± 1.46 months) (Putra et al. 2015) and Madura cattle (21.12 ± 0.16 months) (Kutsiyah, 2017) and Ongole crossbred cattle in Kebumen (26.87 ± 5.51 months) (Rohyan et al. 2016). This was due to differences in maintenance and maintenance objectives carried out by each farmer. The first calving Kuantan-cows on 54.5 ± 1.53 months, it was longer than Madura cattle (31.97 ± 6.43 months), Pesisir cattle (33.83 months) Ongole crossbred cattle (37.15±5.87 months), and Bali cattle (31.92±0.19 months) (Kutsiyah, 2017; Putra et al. 2015; Rohyan et al. 2016; Samberi et al. 2010)

The age of first calving of Kuantan cattle was older than that of other cattle due to late first mating. Likewise, the weaning age of Kuantan cattle (11.5 ± 3.2 months) was older than that of Pesisir cattle (5.57 + 0.31 months) (Putra et al. 2015), Madura cattle (5.06 ± 0.88 months) (Kutsiyah, 2017), PO cattle (3.97 ± 1.10 months) and Bali cattle (4.41 ± 0.32 months) (Samberi et al. 2010). Although the age weaning of Kuantan cattle was high, it did not affect the age of first mating after giving birth 4.8 ± 2.2 months.

The first mating interval after giving birth of Kuantan cattle was shorter than Pesisir cows (5.30+0.51 months) (Putra et al. 2015), the same as PO cattle (4.52±1.59 months) (Rohyan et al. 2016) and longer than Bali cattle (3.53±0.29 months). Likewise, the calving interval of Kuantan cattle (14.74±9.2 months) is not much different from PO cattle (14.32±1.93 months), higher than Bali-cows (13.68±0.51 months) and lower than Pesisir cattle (16.20±0.92 months). These

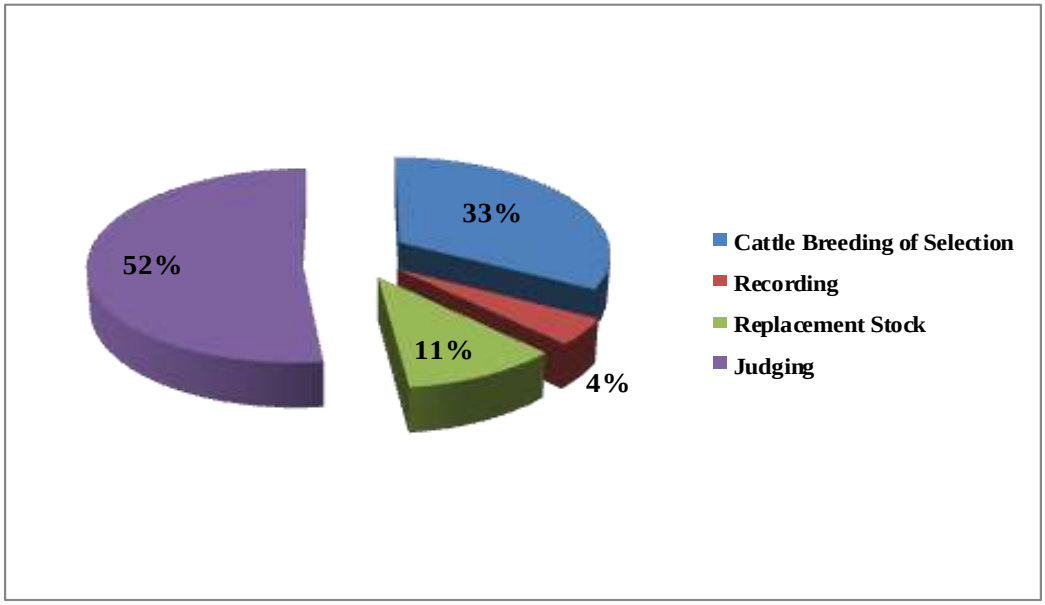

Figure 2. Breed of management carried out by breeders

Table 1 Kuansing land area profile

Usage Type	Large (Ha)	Percentage (%)
Land of paddy fields	17.298	2.94
The yard	51.163	8.69
The moor	59.083	10.03
Plantation land	402.738	68.38
Pasture	1.831	0.31
Temporary land has not been worked on	35.192	5.98
Other	21.682	3.68
Total	588.987	100,00

Table 2. Reproduction characteristics of Kuantan cattle

Description	Average ± DS
First Mating Age (Month)	
Sire	24.5 ±3,03
Mare	45.6 ±1,93
First Calving Age (Month)	54.5 ± 1.53
Conception Period (Month)	9.9 ± 2.5
Calving Interval (Month)	14.74 ± 9.2
Weaning Age (Month)	11.5 ± 3.2
Post-Partum Mating (Month)	4.8 ± 2.2
Mating Method	
Artificial insemination (%)	8.3
Natural mating (%)	92.6
Number of calves dam	
Male (Heads)	111
Female (Heads)	333
Total (Heads)	444
Body condition after parturition	
Fatty (%)	10
Medium (%)	25
Thin (%)	75
Rearing limitation age (years)	
Sire	2.26
Mare	4.31
Respondents amount (People)	121
Dam total on population (Heads)	180
Sample population (Heads)	311
Reproductive Efficiency (%)	102.79

Table 3. The natural increase of Kuantan cattle in Kuansing Regency

Description	Average
Population (heads)	311
Dam population (heads)	180
Dam population on average (%)	57.87
Calving (heads)	44
Male (heads)	11
On dam population (%)	6.1
On average population (%)	3.53
Dam (heads)	33
On dam population (%)	18.33
On average population (%)	10.6
Calving rate	
On dam population (%)	24.44
On average population (%)	14.14
Mortality	
Total (heads)	28
Mortality on population (%)	9.00
Natural Increase	5.14%

Table 4. Population structure of Kuantan cattle in Kuansing Regency

Composition	Total		
	Heads	Animal Unit	(%)
Mature			
Male	36	36	11.58
Female	180	180	57.88
Total	216	216	69.36
Young			
Male	9	4.5	2.89
Female	42	21	13.50
Total	51	25.5	16.39
Calf			
Male calf	11	2.75	3.54
Female calf	33	8.25	10.61
Total	44	11	14.15
Number of respondents (person)	99		
Number of livestock ownership/respondent (heads)	4.32		

Table 5. Mutation of Kuantan cattle in Kuansing Regency

Description	Percentage (%)
Total population (Heads)	1,118
Livestock entry/purchase	
Mature	
Male (%)	5
Female (%)	8
Young	
Male (%)	103
Female (%)	18
Calf	
Male (%)	11
Female (%)	20
Total (heads)	165
The number of cattle entering in population	14.75
Cattle out (%)	
Mature	
Male (%)	34
Female (%)	21
Young	
Male (%)	106
Female (%)	35
Calf	
Male (%)	4
Female (%)	9
Total (Heads)	209
The number of livestock that come out of the population	18.69
Slaughter of cattle (%)	
Mature	
Male (%)	98
Female (%)	24
Total the slaughter of cattle (Heads)	122
The number of cattle slaughtered against in population (%)	10.9

characteristics show that Kuantan cattle have good fertility to be developed as breeding cattle Romjali (2018) states that Indonesian local cattle can reproduce well in conditions of environmental stress that are less supportive than cattle originating from subtropical areas and this value will be an important point in local cattle breeding.

The reproduction efficiency of the Kuantan cattle was 102.79%, higher than PO cattle at 91.8%, Bali cattle 88.38% and Madura cattle 93.21% (Rohyan et al.

2016). This percentage showed that the reproduction of Kuantan cattle was not efficient. The higher the percentage of reproductive efficiency, the age of first mating and age of first cow breed is also high (Suyadi et al. 2014). This value becomes a barometer that livestock reproduction management is not good. The availability of feed and mating system in Kuantan cattle is not so good that their reproduction was inefficient in producing off-springs. This can be seen from the condition of the Kuantan-cows that were thin after giving birth. Proper maintenance management will result in lower reproductive efficiency because adequate intake of energy, protein, vitamins, and minerals will make reproductive efficiency optimal (Bindari et al. 2013).

Natural Increase (NI)

The natural increase value of Kuantan cattle in the Kuansing Regency was 5.14% (Table 4). This value was low because Samberi et al. (2010) stated that the NI value consists of three categories, low if NI ≤ 50%, medium if 51% ≤NI≤80%, high if NI ≥ 81%. This value is lower than some local Indonesian cattle, Pasundan cattle (18.46%) (Said et al. 2017), PO cattle (44.68%) (Rohyan et al. 2016), Pesisir cattle (29.46%) (Putra et al. 2015). This difference is due to the different breeding systems and the livestock environment. This NI value is one measure to see an increase or decrease in a livestock population (Warwick et al. 1983). The low NI value of Kuantan cattle was due to the low birth rate compared to the number of existing cows. Sumadi et al. (2002) state that the NI value is influenced by birth rates and livestock mortality in one area. The low NI of Kuantan cattle showed that the management and handling of productive adult females were not very good, so from 180 productive females, cattle that were used as research samples only 44 adult females gave birth equivalent to 24% of productive females. Some factors that were thought to cause a low percentage of productive adult female cattle were the number of males available was reduced due to slaughtering or sold due to the expends of school children. In addition, slaughtering due to customary celebrations and religious holidays cause the depletion of adult male cattle in the field. In addition, the high mortality rate of livestock causes a reduction in the number of males cattle in the field (Budiarto et al. 2013).

Population structure of Kuantan cattle

The population structure of Kuantan cattle in the Kuansing Regency was dominated by 81.9% female cattle (Table 4). This is an indication that the purpose of raising Kuantan cattle is to produce offspring and is a side job (Syatra et al. 2016). The sex ratio of Kuantan cattle in this study was 1 male to 5 females. This sex ratio is different from Maremma animals in Perugia (1: 1.58) (Fioretti et al. 2020), Pesisir cattle (Putra et al. 2015).

and beef cattle in Poso (Tanari et al. 2011). The large composition of female livestock in one area is one indicator that the area can be used as a breeding center (Sumadi, 2001) and the purpose of maintaining livestock of farmers is to breeding. Total livestock ownership was 4 adult livestock and 1 off-spring. The number of livestock ownership in Kuantan is different from the amount of buffalo ownership in Pariaman regency (2.43) (Putra et al. 2017). This difference is due to different types of livestock and motivation to raise an animal.

Composition of Kuantan cattle mutations

The composition of the Kuantan cattle mutation was the process of movement of animals between the same region or in different areas with the buying and selling process. Data in Table 5 shows that the outgoing cattle (28.9%) were higher than the incoming cattle (14.75). This is one indicator that the region is a livestock producer. The weakness of this study area was that livestock production was not proportional to livestock expenditure so that the natural increase in Kuantan cattle was low. Draining of Kuantan-cows, both male and female. This can be seen from the number of productive female livestock sold. This condition will affect the balance of the population in the next few years because poor livestock was maintained for a longer time.

Net Replacement Rate (NRR)

The number of male and female livestock birth in Kuantan cattle did not meet the needs of substitute animals (male NRR, 50%; female NRR 100.56%) (Table 6). The need for substitute livestock is said to be sufficient if the NRR value> 100% (Sumadi et al. 2002).

The NRR value is used to determine whether the number of livestock birth can cover the need for substitute livestock so that the population remains balanced. NRR value is closely related to NI value. The greater the value of NI, the greater the NRR value. The low NRR in Kuantan bulls showed that there was a depletion of Kuantan bulls in the Kuansing regency. This was because of the availability of a bull to replace only 0.5 times the need. This condition can be stated that the Kuansing regency lacks male cattle. While the availability of female Kuantan cows to replace only 1 time. This value is better than the value of bulls (Sumadi et al. 2002). The low NRR value of this

Table 6. The net replacement rate for Kuantan cattle in Kuantan Singing regency

Description	Percentage (%)
Replacement stock necessary (%)	
Male	18
Female	41.76
NRR	
Male	50
Female	100.56

Table 7. Estimated of Kuantan cattle output in the Kuantan Singingi regency in 2018

Description	Percentage (%)
The rest of the replacement (young)	
Male	12,55
Female	-23.43
Availability Replacement	
Male	18
Female	41.76
Culling cattle	
Male	18
Female	41.76
Total Output	48.88

Table 8. Population dynamics of Kuantan cattle

Years	Population	Change in Population (%)
2014	1179	-
2015	1176	-0.26
2016	1118	-5.19
2017	1218	8.21
2018	1278	4.69
2019	1265	-1.03

Kuantan cattle in the Kuansing regency is an indication that the Kuansing area cannot be called the source area of the Kuantan cattle.

The NRR of Kuantan cattle in this study was lower than the NRR of Pasundan cattle (807%) (Said et al. 2017), PO cattle in Kebumen were 253% for females cattle and 1,207% for males cattle (Rohyan et al. 2016) and not much different from Madura cattle on Sapudi island 96% NRR for male cattle, 126% NRR for female cattle) (Kutsiyah, 2017). This difference was due to differences in management, food availability and socio-economic community (Warwick et al. 1983).

Kuantan cattle output

The output of Kuantan cattle (48.88%) (Table 7) greater than the NI value of Kuantan cattle (5.14%). This was an indicator that there was no increase in the population of Kuantan cattle in the Kuansing regency (Sumadi, 2001). The development of the population of Kuantan cattle in the Kuansing regency has begun to be disrupted so that for a while the region was not able to produce Kuantan cattle. For this reason, it is necessary to manage breeding patterns by increasing birth rates, reducing mortality, shortening maintenance time and fattening the rejected animals to prepare substitute livestock for parent-stock. The negative residual replacement in male cattle shows that there was a tendency for people to sell or slaughter cattle before two years old (Putra et al. 2015). The output of Kuantan cattle was higher compared to Bonsamara cattle in Brazil by -3.58% (Santana et al. 2012), Buffalo in Brazil by 12.5% (Ferraz et al. 2015), Bali cattle in Papua by 13.11% (Samberi et al. 2010), Pesisir cattle by 20.25% (Putra et al. 2015) and Madura cattle 30.75% (Kutsiyah, 2017). This difference was due to different regional conditions resulting in different feed quality, maintenance systems and socioeconomic (Budiarto et al. 2013).

Population dynamics of Kuantan cattle

The population dynamics of Kuantan cattle in the Kuansing Regency from 2014 to 2018 was only 2.84% (Table 8) (Department of Animal Husbandry and Animal Health of Riau Province, 2019). This value was not significant to increase the livestock population when compared to the number of available parent-stock.

The prediction population of Kuantan cattle for 2020 to 2023 with regression polynomial, $Y = -2.0544x^3 + 30.617x^2 - 105.42x + 1259.5$, with $R^2 = 0.82$ (Figure 3). The amount of Kuantan cattle availability was smaller than the number of Kuantan cattle needed so this indicates that Kuansing Regency is not yet suitable as a source of Kuantan cattle. The population dynamics is a reflection of the reproductive appearance of Kuantan cattle. The low reproductive performance will reduce the increase in livestock population (Gunawan et al. 2011). The low dynamic population will result in generation loss (Makina et al. 2015). One effort to improve livestock reproduction is by selecting livestock germs from both prospective mothers and prospective males. This analysis of population dynamics is needed to determine the amount of livestock expenditure from the area so that there is no

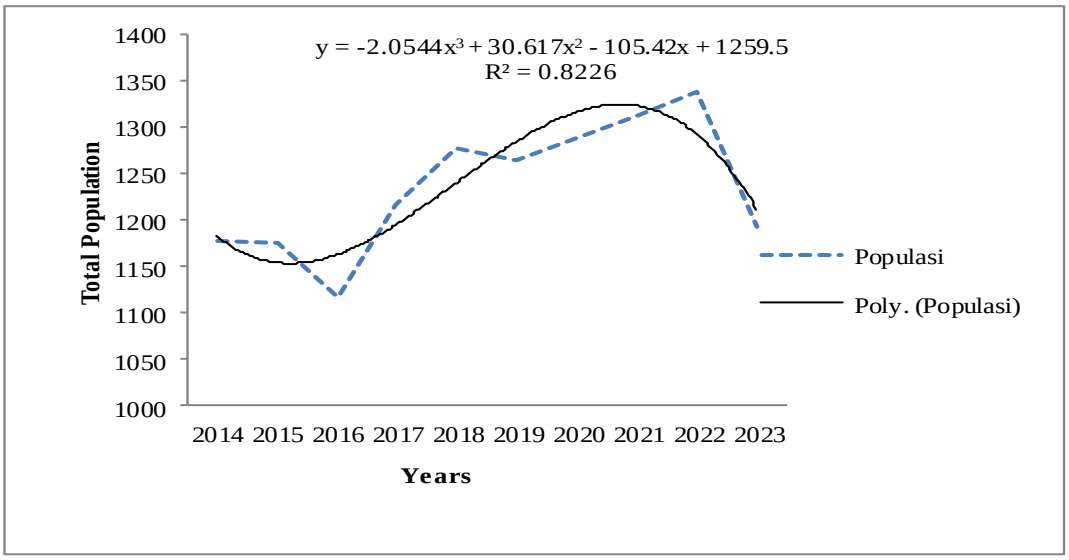

Figure 3. Prediction population n of Kuantan cattle in Kuantan Regency, Indonesia

depletion of the population (Fioretti et al. 2020). The population dynamics of Kuantan cattle were different from those of native cattle (Sylhet) Bangladesh (Koirala et al. 2011); Pasundan cattle (Said et al. 2017) and breed Ongole cattle (Rohyan et al. 2016).

CONCLUSION

The reproductive efficiency of Kuantan cattle was 1.04%, natural increase 5.14%, male and female counterpart 1: 5, the NRR of male 50%, and female 100.56%, total cattle out 18.69% and total livestock intake 18.69%, output 48.88% and estimated population dynamics 2.85%. It is recommended not to release Kuantan cattle in the next 5 years to maintain population balance.

ACKNOWLEDGMENT

Thanks to the Rector of UIN Suska Riau for providing funding support in this study, the farmers who became the study sample, the Departement Animal Husbandry and Agriculture Kuansing Regency and field officers.

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Novel Mutation of Exon 5 Prolactin Gene in IPB-D1 Chicken

Rohmah L[1,2], Darwati S[2], Ulupi N[2], Khaerunnisa I[3], Sumantri C[2]

[1]*Graduate School. Department of Animal Production and Technology, IPB University, Bogor 16680*
[2]*Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680*
[3]*Indonesian Institute of Sciences, Sasana Widya Sarwono (SWS), Jl. Jend. Gatot Subroto 10, Jakarta 12710*
E-mail: lailatulrohmahh@gmail.com

(received 19-06-2020; revised 29-09-2020; accepted 15-10-2020)

ABSTRAK

Rohmah L, Darwati S, Ulupi N, Khaerunnisa I, Sumantri C. 2020. Mutasi baru gen prolaktin ekson 5 pada ayam IPB-D1. JITV 25(4): 173-181.DOI: http://dx.doi.org/10.14334/jitv.v25i4.2525

Gen prolaktin (PRL) merupakan salah satu gen yang mengontrol sifat mengeram dan produksi telur pada ayam petelur. Sifat mengeram akan menurunkan produksi telur dan mengganggu sistem reproduksi pada ayam lokal. Tujuan dari penelitian ini yaitu mengidentifikasi keragaman gen prolaktin pada ayam IPB-D1 dengan menggunakan metode *direct sequencing*. Analisis keragaman pada gen prolaktin ekson 5 dilakukan pada 46 sampel DNA ayam IPB-D1 yang merupakan koleksi Divisi Genetika dan Pemuliaan Ternak, Fakultas Peternakan IPB. Sekuens DNA sebagai acuan untuk merancang primer ekson 5 didapatkan dari *National Center for Biotechnology Information* (NCBI) dengan kode akses GenBank AF288765.2. Ekstraksi DNA dilakukan dengan menggunakan teknik *phenol-chloroform*. Amplifikasi DNA menghasilkan produk PCR dengan ukuran 557 pb. Pada penelitian ini dihitung frekuensi genotipe, frekuensi alel, dan nilai heterozigositas serta keseimbangan Hardy-Weinberg. Hasil penelitian menemukan 5 SNP pada ekson 5, yaitu g.7823A>G, g.7835A>G, g.7886T>A, g.8052T>C, dan g.8069T>C. Seluruh SNPs bersifat polimorfik dan berada dalam keseimbangan Hardy-Weinberg kecuali g.8052T>C. Mutasi basa g.7823A>G, g.7835A>G, g.8052T>C merupakan mutasi *synonymous* yang tidak mengubah asam amino, sedangkan mutasi basa g.7886T>A dan g.8069T>C merupakan SNP *non-synonymous* yang mengubah asam amino sehingga dapat dijadikan sebagai kandidat *marker assisted selection* sifat produksi telur pada ayam IPB-D1.

Kata Kunci: Ayam IPB-D1, Gen Prolaktin, Mutasi, SNP

ABSTRACT

Rohmah L, Darwati S, Ulupi N, Khaerunnisa I, Sumantri C. 2020. Novel mutation of exon 5 prolactin gene in IPB-D1 chicken. JITV 25(4): 173-189. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2525

The prolactin gene (PRL) is a gene that controls the incubation and egg production in laying chickens. The nature of incubation will reduce egg production and disrupt the reproductive system in local chickens. The purpose of this study was to identify the polymorphism of prolactin genes in IPB-D1 chickens using the direct sequencing method. The polymorphism of the exon 5 prolactin gene was carried out on 46 samples of IPB-D1 chicken DNA which was a collection of the Division of Animal Genetics and Breeding, Faculty of Animal Science IPB. DNA sequences as a reference for designing exon 5 primers were obtained from the National Center for Biotechnology Information (NCBI) with the GenBank access code: AF288765.2. DNA extraction was carried out using the phenol-chloroform technique. DNA amplification resulted in a PCR product with a size of 557 bp. In this study, the genotype frequency, allele frequency, heterozygosity value and Hardy-Weinberg equilibrium were calculated. The results of the study found 5 SNPs in exon 5, namely g.7823A>G, g.7835A>G, g.7886T>A, g.8052T>C, and g.8069T>C. All SNPs are polymorphic and in Hardy-Weinberg equilibrium except g.8052T>C. The g.7823A>G, g.7835A>G, g.8052T>C SNPs are synonymous mutations that do not change amino acids, while the g.7886T>A and g.8069T>C SNPs are non-synonymous that change amino acids. Both g.7886T>A and g.8069T>C SNPs are potential as a marker assisted selection for the characteristics of egg production in IPB-D1 chickens.

Key Words: IPB-D1 Chicken, Mutation, Prolactin Gene, SNP

INTRODUCTION

In 2019, the native chicken population in Indonesia was approximately 8.37% of the national chicken population (DJPKH 2019). This contributed 6.01% of the total national meat production in 2018 which decreased compared to those in 2017 (DJPKH 2019). The low productivity of native Indonesian chicken eggs is one of the challenges of the native Indonesian chicken industry. Sinurat et al. (1992) in: Hidayat & Asmarasari (2015) reported that the egg production of native chicken that is extensively reared is up to 30.20 eggs per chicken per year. Meanwhile, with an intensive maintenance system, the egg production of native

chicken eggs reaches 80.30 eggs per chicken per year (Sinurat et al. 1992). One of the solutions to increase the productivity of local chickens is the invention of a novel crossbreed chicken, namely IPB-D1 (Ministry of Agriculture Reg No. 693/KPTS/PK.230/M/9/2019). IPB-D1 chicken is a result of crossbreeding between F1 ♂Pelung x ♀Sentul and F1 ♂Kampung x ♀Parent Stock Cobb. The superiority of this chicken is its fast growth, which reaches 1.18 ± 0.2 kg (male) or 1.04 ± 0.12 kg (female) slaughter weight at the age of 10-12 weeks. In addition, this chicken also resistant to New Castle disease (Sumantri & Darwati 2017).

The IPB-D1 composite chickens also showed the superiority of reproduction and egg production. Cholifah (2018) reported that crossbreeding between pelung, sentul, Kampung, and broiler chickens produced higher fertility, higher hatchability, and lower mortality than Kampung chicken. In addition, the crossbreeding between Kampung chicken with broilers has a low incubation capacity that is able to produce a higher egg production. The production and reproduction of the IPB-D1 chicken in the 3rd generation, in this case, is explained by Sumantri & Darwati (2017). It is mentioned that the age of first laying eggs is 24 weeks with bodyweight of 1.80±0.23 kg. Meanwhile, egg production of the chicken is 45.20% with the egg weight which is 33.50±0.97 grams. Furthermore, the egg fertility of IPB-D1 chicken is 84.25% with 64.70% of hatchability (Sumantri & Darwati 2017). In further research, Habiburahman et al. (2020) mentioned that the egg production of this chicken increased to 49.22% in the 7th generation. The increase in egg production of IPB D-1 G7 chickens was due to the genetic percentage of broilers as much as 25%. The 25% genetic contribution of purebred chickens has led to an increase in egg production of IPB D-1 chicken 7th generation compared to local chickens in general.

To overcome the low production of native chicken eggs among other things, influenced by the nature of the incubation, it can be done by reducing or eliminating the broody nature of the chicken. Molecular selection can be done quickly and as early as possible through the marker assisted selection of natural brooding. The prolactin gene (PRL) is a gene that controls the incubation and egg production in laying chickens. Prolactin is a single chain polypeptide hormone that belongs to a family of growth hormone genes and is generally synthesized in the anterior pituitary in all vertebrate animals (Sharp et al. 1979). In aves, it has been reported that prolactin has many important roles in the regulation of physiological processes, including egg production, stimulating and maintaining the nature of the incubation, osmoregulation, immune-modulation, function and development of gonad cells (Plant & Zeleznik 2014). Sherwood et al. (2005) mentioned that prolactin is a gene that controls the nature of brooding because prolactin is an important part of the functioning of neuroendocrine which stimulates the occurrence of incubation. This incubation event is caused by the prolactin promoter functioning to activate the initial transcription of the prolactin gene expression. Mutations in the prolactin promoter are reported to affect the activity of the prolactin gene in the incubation process (Sartika 2005). In this case, the prolactin gene (PRL) has a major role in the nature of the incubation and egg production in chickens. Therefore prolactin gene can be used as genetic markers in Indonesian local chicken selection programs, especially in IPB-D1 chickens. This study aims to identify the polymorphism of prolactin genes (PRL) in IPB-D1 chickens.

MATERIALS AND METHODS

Animals

All procedures performed in this study were approved by the Animal Care and Use Committee (ACUC) of IPB University (ACUC No: 163-2019 IPB). A number of 46 IPB-D1 chickens (24 weeks-old, hen) were collected from Genetics and Animal Breeding Division, Faculty of Animal Science, IPB. All chickens were kept under uniform conditions with a uniform feed of protein and energy balance. Feed and water were given *ad libitum*. Blood samples were taken from the pectoralis vein in the wing area. From this process, a total of 1.0-1.5 ml samples were taken using a syringe. Blood is put into a tube containing K3-EDTA *(ethylenediamine tetraacetic)* anticoagulant and then stored in a refrigerator at 4 ^{o}C until it is ready for DNA extraction.

Primer designing

The primer sequences were designed using the Primer3 and BLAST Primer applications from NCBI to obtain the primer target according to Ye et al. (2012). It also used the Primer Stats application for primer compatibility test. The length of the PCR product was 557 bp and it is located in exon 5. Sequential data for the primer design were obtained from the National Center for Biotechnology Information (NCBI) with the GenBank access code: AF288765.2. The primer sequence used in this was the author's design with the following primers. The forward primer sequence was (F): 5'-TGGAGGAGGCCAAAAAGAGATG-3' and reverse primer sequence was (R): 5'-GCAGCCCACAGGTACTTAGC-3'.

DNA isolation

Genomic DNA was extracted by using Phenol-chloroform technique (Sambrook & Russell 2001) and

modified by adding cells buffer lysis (250 µl 1 x STE, 40 µl SDS, and 10 µl proteinase-K). The DNA was purified by adding 40 µl 5M NaCl, 400 µl phenol, and 400 µl CIAA (Chloroform Iso Amyl Alcohol) and precipitated by using 40 µl 5M NaCl and 800 µl ethanol absolute. The precipitation was washed once by adding 800 µl of 70% ethanol and centrifuged with a speed of 12.000 rpm for 5 min. The ethanol was discarded and evaporated, then the precipitated DNA was dissolved in 100 µl of 80% TE (Elution buffer).

DNA amplification

The amplification of PRL gene fragments was done by PCR machine. The DNA sample to be amplified was transferred into a 0.2 ml tube with a sample volume of 0.5 µl. After the sample was added the 14 µl premix containing 0.4 µl primers, 12.5 µl Green Master mix, and 11.1 µl Nuclease Free Water. Then the sample was homogenized and put into the ESCO Swift Maxi Thermal Cycler machine. The PCR technique was carried out through 3 stages. The first stage was the cycle of the temperature predenaturation process of 95ºC for 5 minutes and the second stage, it was carried out 35 cycles consisting of denaturation (95ºC) for 10 seconds, annealing (57ºC) for 20 sec, and extension (72ºC) for 30 seconds. Meanwhile, the third stage was in the form of a final extension (72ºC).

Direct sequencing and genotyping

Direct sequencing was carried out from two directions, forward and reverse. Sequence products were obtained by using services from 1st Base Selangor, Malaysia. The sample sequencing results were then verified through alignment by multiple alignment ClustalW in MEGA-X (Kumar et al. 2018). The alignment of IPB-D1 chicken samples was carried out using BioEdit programs to identify the polymorphism in this study (Alzohairy 2011). IPB-D1 chicken sequences were compared with GenBank from the National Center for Biotechnology Information (NCBI) with access code of the GenBank AF288765.2. The sequencing chromatogram results are scanned using FinchTV programs to identify heterozygous of the polymorphic positions which is marked by a double peak on the chromatograms (Treves 2010).

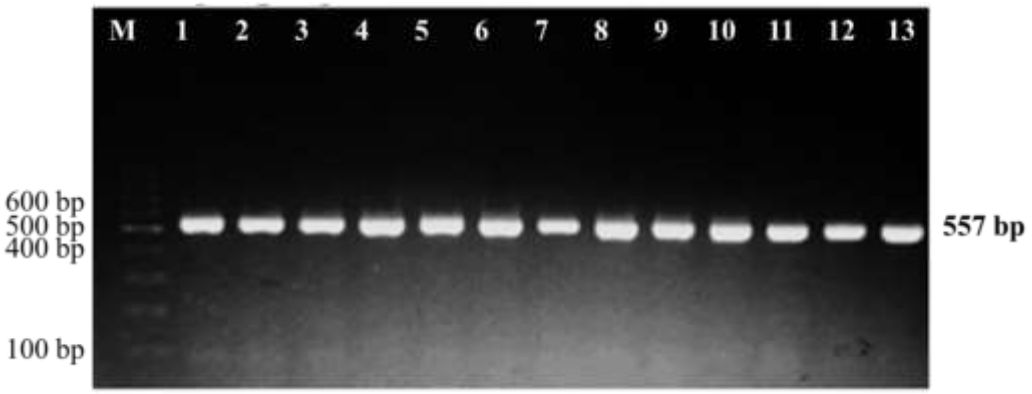

Figure 1. PCR amplification product of PRL gene (557 bp); M= DNA ladder 100 bp; 1-13 = IPB-D1 chicken samples

Data analysis

Genetic diversity analysis was performed by calculating genotype frequency and allele frequency. In addition, the chi-square value and heterozygosity values were also calculated. The formula for calculating allele frequencies and genotype frequencies according to Nei and Kumar (2000) is as follows.

$$X_i = \frac{(2N_{ii}+N_{ij})}{(2N)}; \qquad X_j = 1\text{-}X_i$$

Where, Xi = Frequency of the i^{th} allele; Xj = Frequency of the j^{th} allele; Nii = Number of samples with genotype ii; Nij = Number of samples with ij genotypes; and N = Number of samples.

Meanwhile, for the genotype frequency was calculated using the following formula.

$$X_{ii} = \frac{N_{ii}}{N}x100\% \qquad X_{ij} = \frac{N_{ij}}{N}x100\% \qquad X_{jj} = \frac{N_{jj}}{N}x100\%$$

Where, Xii = Frequency of the ii^{th} genotype; Xij = Frequency of the j^{th} genotype; and Xjj = Frequency of ij^{th} genotype.

Furthermore, genetic diversity was calculated using the frequency of observed heterozygosity (Ho) and expected heterozygosity (He) was calculated using the Weir (1996) formulas as follows.

$$H_o = \sum_{i\neq j} \frac{n_{ij}}{N} \; ; \; H_e = 1 - \sum_{i=j}^{q} X_i^2$$

Where, Ho= heterozyegosity observations (population); He = value of expected heterozygosity; nij= number of heterozygous individuals; N = number of individuals observed; Xi = allele frequency; and q = number of alleles.

Genotype frequency deviations that arise from Hardy Weinberg's equilibrium were analyzed using the chi-square test (x^2) based on the Nei and Kumar (2000) formula as follows.

$$X^2 = \sum_{i=1}^{n} \frac{(O-E)^2}{E}$$

Where, X^2 = chi-square test; O = Frequency of observed sample genotypes; and E = frequency of expected genotype.

RESULTS AND DISCUSSION

Discovery single nucleotide polymorphisms of exon 5 prolactin gene

The results of prolactin gene sequencing are shown in Figure 2. Sequencing results indicated the same mutation position based on Osman et al. (2018), Erehehuara (2003), and Li et al. (2013), i.e. g.8052T>C. Furthermore, 4 novel of mutation points were also found, namely g.7823A>G, g.7835A>G, g.7886T>A, and g.8069T>C.

Single Nucleotide Polymorphism (SNP) is a form of differences in genetic material variation between two individuals in the form of a single nucleotide in a series of DNA nucleotide base sequences at specific locations in the genome (Murray et al. 2014). The mutation point positions on exon 5 found in this study are g.7823A>G, g.7835A>G, g.7886T>A, g.8052T>C, and g.8069T>C which all of them are classified as substitution mutations. In Figure 3, a partial sequence of prolactin gene mutations is described. Nucleotide base substitution mutations are divided into transition and transversion substitution mutations. The base mutations of g.7823A>G, g.7835A>G, g.8052T>C, and g.8069T>C classified as a transition mutation, which changes purine with other purine (A>G) or pyrimidine with other pyrimidine (C>T). Whereas transversion are purine substituted with pyrimidine bases or vice versa (Luo et al. 2016). Transition mutations are common, although most of them repaired by various proof-reading function (Dubey 2014).

Adenine-Thymine in DNA conformation has two hydrogen bridges (Muladno 2010). Hydrogen bonds are non-covalent interactions that have small free energy of 2-6 kj mol^{-1} in water, so that the strength of a weak bridge will be easily split and put back together (Ferst 2017). Changes in the structure of hydrogen bonds can affect DNA transcription and changes in the structure or shape of protein molecules produced. According to Luo et al. (2016), transversion mutations are more influential on changes in the structure and function of regulation of a gene than with transition mutations.

Each mutation that appears will affect 1 or more roles of the encoded protein. The role of proteins that may be affected by mutations is stability or folding protein, ligand binding protein, catalysis, regulation with allosteric and other mechanisms as well as post-translational protein modification (Nagasundaram et al. 2015). Overall changes in amino acids from the prolactin gene mRNA sequence as a result of translation are presented in Table 1.

Table 1. Amino acids changes from the prolactin gene mRNA sequence as a result of translation

SNPs Position	**A**mino Acids Changes		
g.7823A>G	Lysine	>	Lysine
g.7835A>G	Leucine	>	Leucine
g.7886T>A	Aspartate	>	Valine
g.8052T>C	Isoleucine	>	Isoleucine
g.8069T>C	Leucine	>	Serine

The g.7823A>G (Lys>Lys), g.7835A>G (Leu>Leu) and g.8052T>C (Ile>Ile) SNPs are synonymous mutations that do not change amino acids. Synonymous mutations can encode sequences with the same amino acid composition, but the structure and function of the protein can change by influencing the mechanism of RNA transcription, mRNA structure, and translational speed (Supek et al. 2014; (Sauna & Kimchi-Sarfaty 2011).

The g.7886T>A (Asp>Val) and g.8069T>C (Leu>Ser) SNPs are non-synonymous mutations that convert amino acids. The g.7886T>A changes the aspartate amino acid to valine. The structure of these amino acids was changed where valine is one of three branched-chain amino acids (the others are leucine and isoleucine) that enhance energy, increase endurance, and aid in muscle tissue recovery and repair (NCBI 2020). This group also lowers elevated blood sugar levels and increases growth hormone production in chicken (Nascimento et al. 2016). The g.8096T>C changes the leucine amino acid to serine. This amino acid changes involved in the functioning of RNA and DNA, in the muscle formation as well as in the maintenance of a proper immune system (NCBI 2020b).

Based on the mechanism of the prolactin gene action, mutations in DNA sequences can change the structure of the resulting protein so that it can affect the signaling pathway of the prolactin gene, especially in the process of attaching prolactin to its receptors. The prolactin pathway is initiated by attaching prolactin to its receptors. Prolactin receptors will induce signals and binds to the signal transduction also activates the protein transcription factor (Radhakrishnan et al. 2012).

Stat proteins (signal transducers and activators of transcription) will dissociate with their receptors and

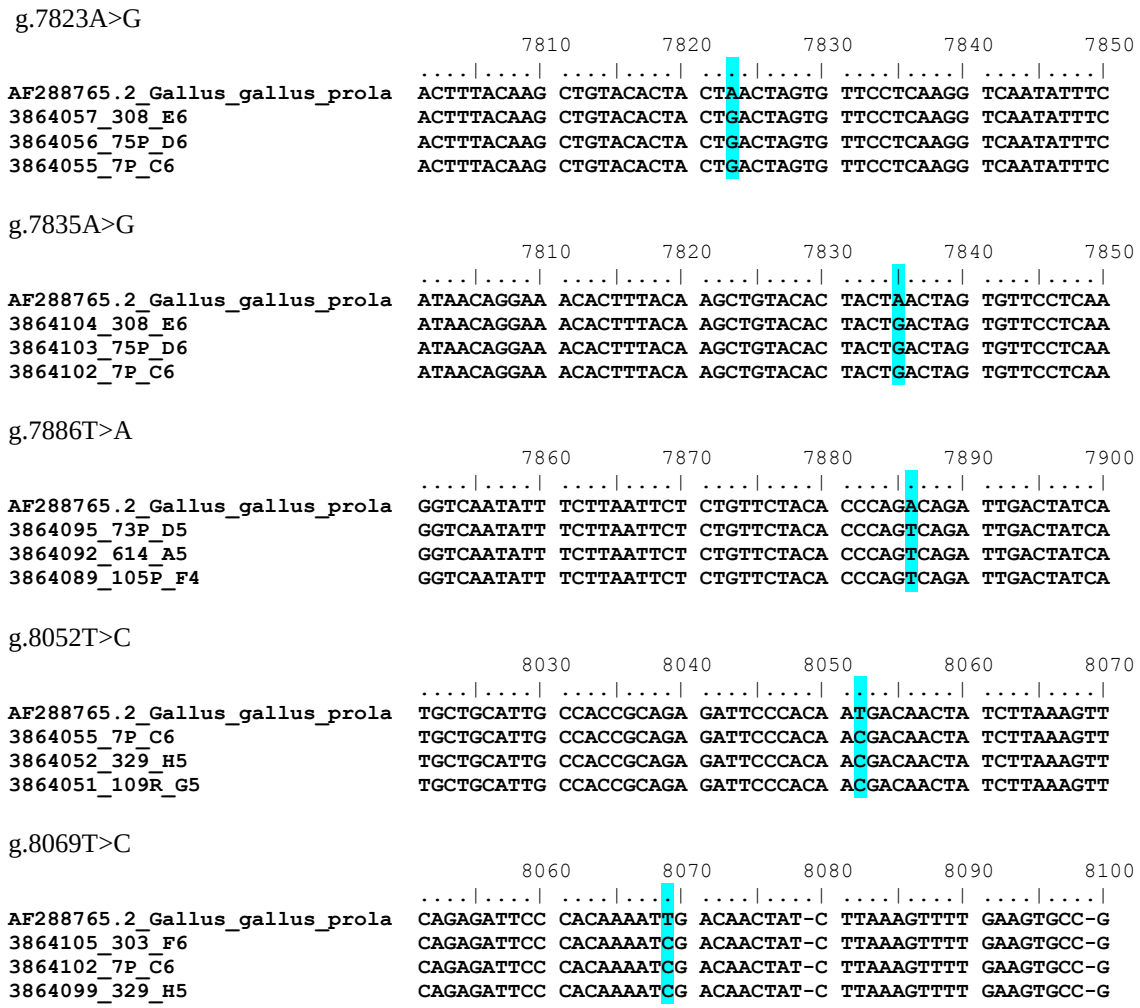

Figure 2. Alignmented of prolactin gene at exon 5 in IPB-D1 chicken showing 5 Single Nucleotide Polymorphisms (SNPs). Blue highlights show mutation point of each SNP

translocation will occur to the nucleus to bind to the target gene for the promoter prolactin. Attachment to the regulatory part of prolactin will regulate the active and inactive mechanism of the prolactin gene (Radhakrishnan et al. 2012). Thus, when a DNA sequence mutation occurs that changes the structure of the protein, it can cause inactivation of the prolactin gene

Genotype frequency, allele frequency, and prolactin gene heterozigosity

In this study, the chi-square analysis (χ^2) was conducted to determine whether the population is in Hardy-Weinberg equilibrium. In addition, this analysis was also carried out since in an experiment, often the results of crossing carried out offsprings that are not in accordance with Mendel's law. The population is mentioned to be in equilibrium if the calculated value of χ^2 is smaller than χ^2 table. A population that is in equilibrium means that the population has not yet experienced mutations, migration, directed marriages, selection, and large sample sizes (Castle 1903).

Heterozygosity is a parameter used to measure the level of genetic diversity in a population based on the allele frequency of each locus (Wang et al. 2015). Objective heterozygosity (Ho) is the average number of individuals with polymorphic locus based on observations, while expected heterozygosity (He) is an estimate of genetic diversity based on the results of allele frequency calculations. The observed heterozygosity value is greater than the expected heterozygosity value. These findings indicate that the population is diverse. High genetic diversity has heterozygosity values of more than 0.50 (Karabağ et al. 2016). The results of genotype frequency, allele frequency, heterozygosity, and chi-square analysis (χ^2) of the prolactin gene at various locus are presented in Table 2.

Genetic diversity in populations is illustrated by 3 diversity indices, namely the number (percentage) of polymorphic loci in the population, average.

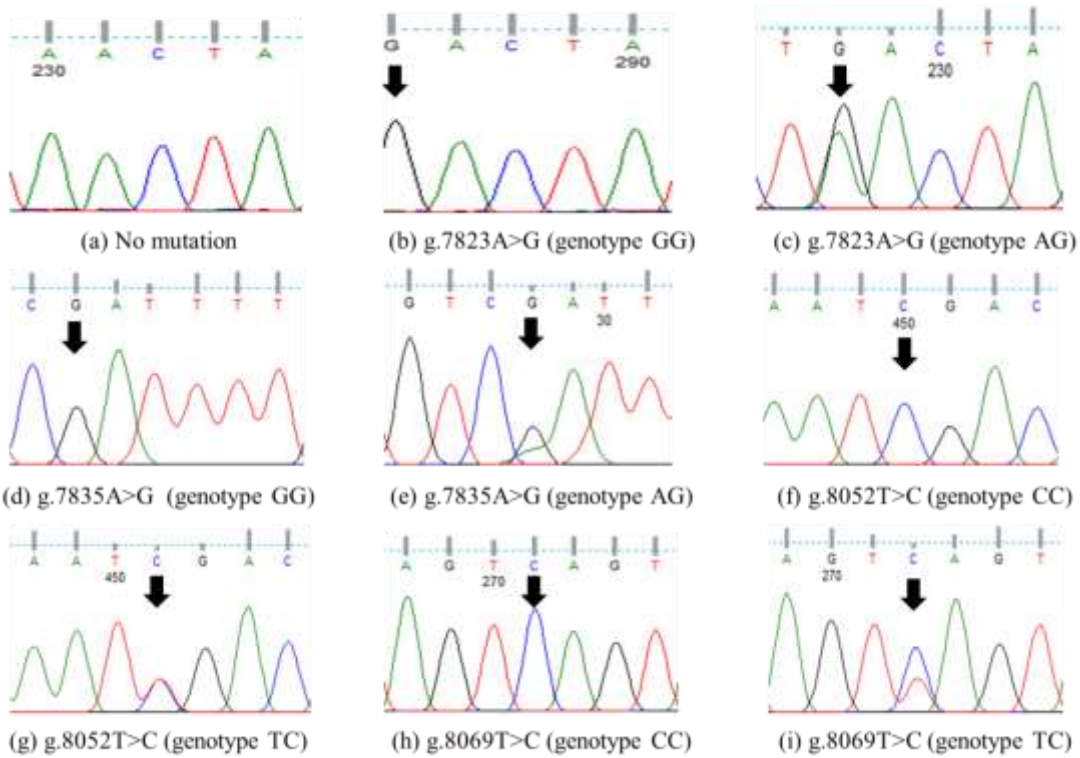

Figure 3. Partial sequencing maps of exon 5 prolactin gene showing transition mutations in IPB-D1 chicken. Arrows show mutation point.

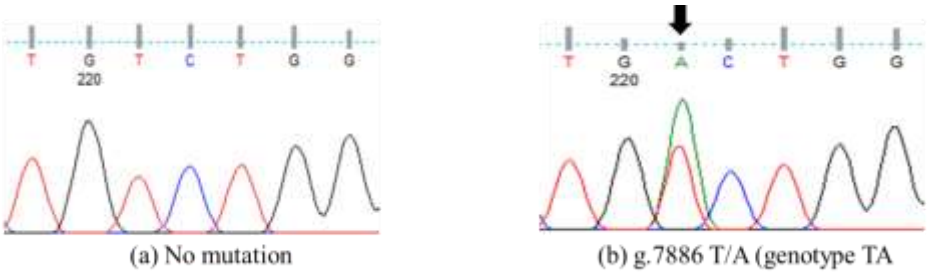

Figure 4. Partial sequencing maps of exon 5 prolactin gene showing transversion mutation in IPB-D1 chicken. Arrow shows mutation point.

Table 2. Genotype frequency, allele frequency, heterozygosity, and chi-square test (χ^2) prolactin gene values at various locus in IPB-D1 chicken

SNP	N	Genotype frequency (n)			Allele frequency		χ^2	H_o	H_e
g.7823 A>G	46	AA 0.50 (23)	AG 0.37 (17)	GG 0.13 (6)	A 0.68	G 0.32	0.95^{ns}	0.43	0.37
g.7835 A>G	46	AA 0.22 (10)	AG 0.57 (26)	GG 0.22 (10)	A 0.50	G 0.50	0.78^{ns}	0.57	0.50
g.7886 T>A	46	TT 0.91 (42)	TA 0.09 (4)	AA 0.00 (0)	T 0.96	A 0.04	0.14^{ns}	0.09	0.08
g.8052 T>C	46	TT 0.69 (32)	TC 0.22 (10)	CC 0.09 (4)	T 0.80	C 0.20	4.27^{*}	0.22	0.31
g.8069 T>C	46	TT 0.26 (12)	TC 0.57 (26)	CC 0.17 (8)	T 0.46	C 0.54	0.89^{ns}	0.57	0.50

N = total sample; (n) = number of samples on the genotype. *=significantly different at P<0.05 ($x^2_{0.05}$=3.84), ns=non significant

heterozygosity at Hardy-Weinberg equilibrium, and allele frequencies (Nei & Kumar 2000). Allele frequency is a measure of the relative frequency of an allele in a population that shows genetic diversity. An allele can be said to be polymorphic if it has the same frequency or less than 0.99 (Hartl & Clark 2000).

The results of the study found five points mutation on exon 5: i.e g.7823A>G, g.7835A>G, g.7886T>A, g.8052T>C, and g.8069T>C. The g.7823A>G and g.7835A>G SNPs produced 3 genotypes: AA, AG, and GG. The g.7823A>G had a higher AA (50.00%) genotype frequency compared to the AG (36.96%) and GG (13.04%) (Table 2). The high frequency of the AA genotype resulted in a higher range of the A allele frequency (0.68) compared to the G allele frequency (0.32) which indicated that the locus was polymorphic. Allele frequency is a measure of the relative frequency of an allele in a population that shows genetic diversity. An allele is classified as polymorphic if it has a frequency equal to or less than 0.99 (Hartl & Clark 2000). The results of the Hardy-Weinberg equilibrium test showed that the results were not significantly different (P> 0.05) or were in the Hardy-Weinberg equilibrium. This result is in line with the results of the heterozygosity value in IPB-D1 chickens which showed that the observed heterozygosity value (Ho) was higher than the value of expected heterozygosity (He), which means that the population that has mutations at the locus g.7823A>G varied.

The g.7835A>G locus also showed polymorphic properties in IPB-D1 chickens. The frequency of the AG genotype (57.00%) was higher than the AA (22.00%) and GG (22.00%) genotypes (Table 2), resulting in balanced A (0.50) and G (0.50) allele frequency. The results of the x^2 were in the Hardy-Weinberg equilibrium (P>0.05) which indicated that the observed heterozygosity value was higher than the expected heterozygosity value, which meant that the population that had mutations at locus g.7835A>G varied.

SNP at locus g.7886T>A was found only 2 genotypes, namely TT (91.30%) and TA (8.70%). The high frequency of the TT genotype resulted in a higher range of the T allele (0.96) than the A allele (0.04), and the frequency magnitudes of these two alleles showed polymorphic. The genotype frequency of SNP locus g.7886T>A based on the chi-square value (χ^2), the IPB-D1 chickens were in Hardy-Weinberg equilibrium (P>0.05). This is reinforced by the results of the calculation of the heterozygosity value obtained in IPB-D1 chickens, that the observed heterozygosity value is greater than the expected heterozygosity value which means that the population at the locus is polymorphic.

SNP at locus g.8052T>C found 3 kinds of genotypes: TT (69.57%), TC (21.74%), and CC (8.70%) (Table 2). The high frequency of TT genotypes resulted in higher T allele (0.80) compared to C allele (0.20) so that the g.8052 locus was also polymorphic in IPB-D1 chickens. The results of the Hardy-Weinberg balance test with the chi square test (χ^2) indicate that they are in Hardy-Weinberg equilibrium (P>0.05). The g.8069T>C locus also produced 3 genotypes: TT (26.09%), TC (56.52%), and CC (17.39%) (Table 2). The frequency of the C allele (0.54) is greater than the T allele (0.46) and shows the polymorphism of the prolactin gene in IPB-D1 chickens. Allele frequencies in the five SNPs identified have a value $\leq$0.99. Therefore it can be concluded that the prolactin gene in chicken IPB-D1 is polymorphic. An allele is classified as polymorphic if it has an allele frequency $\leq$0.99 (Hartl & Clark 2000).

In this current study, based on allele frequencies, the PIC values calculated the result ranged from 0.08 (g.7886) to 0.50 (g.7835 and g.8069) and the average PIC values were calculated from overall locus as 0.37. DNA markers showed an average 0.25 < PIC value < 0.50, which confirms that the marker is reasonably informative (Botstein et al. 1980). The Hardy-Weinberg equilibrium test results, which have been carried out, are known to be in Hardy-Weinberg equilibrium (P> 0.05) which means that the observed heterozygosity values are higher than the expected heterozygosity values. From these findings, it can be said that the population that has mutations at the locus is diverse. This is possible because of several factors, including the lack of optimal selection efforts for chickens from IPB-D1. Castle (1903) states that a large population will not change from one generation to another if there is no selection, migration, mutation, and genetic drift.

The SNP of the exon 5 of the prolactin gene has actually been previously studied and found SNP at position g.8052T>C in local Chinese chickens, namely Qinyuan Patridge chickens and in recessive white chickens (Erehehuara 2003; Li et al. 2013). In addition, non-synonymous SNP position g.7921C>T was found which changed the amino acid Ser into Pro and SNP synonymous at position g.8187C>T in the F15 hubbard chicken (Osman et al. 2018). The results of SNP identification at g.8052T>C in this study are in accordance with the SNP findings of Li et al. (2013) and Erehehuara (2003). Li et al. (2013) showed in the results of his study that the position of SNP g.8052T>C was significantly related to the first age of the chicken laying eggs and the amount of egg production at the age of the first 300 days laying eggs. The g.8052T>C SNP located in the coding region of the gene, but does not change the amino acid sequence, although the egg production traits between two chicken breeds were significantly different, the allele frequencies of the two sites in both breeds were close and only significant genotypic effects existed. The association analysis suggested that there were significant associations

between T8052C and G8113C genotypes of PRL gene and the egg production traits of AFE and EN 300. Moreover, the breed effect and the interaction effect between breeds and genotypes are not significant, and therefore, it was concluded that there may be a relationship between these two SNP sites and egg production traits in chickens. In this study, the H2H3 diplotype was also found to be associated with more egg production traits than other diplotypes (H2H2, H2H4 and H3H3), indicating that H2H3 diplotype may be the most advantageous haplotypes for egg production. Erehehuara (2003) also mentioned that SNP g.8052T>C and g.8113G>C greatly influenced egg production in white leghorn and brown hy-line chickens, that the frequency of A1A1 genotype, was 1.0 and 0.84 in white leghorn and brown hy-line egg layers, respectively.

CONCLUSION

Five SNPs have been found in the exon 5 of prolactin gene fragments in IPB-D1 chicken. The five SNPs including g.7823A>G, g.7835A>G, g.7886T>A, g.8052T>C, and g.8069T>C. The g.7823A>G, g.7835A>G, and g.8052T>C are synonymous mutations, whereas g.7886T>A and g.8069T>C are non-synonymous mutation which alters change amino acids. Both g.7886T>A and g.8069T>C can be used as a candidate of marker-assisted selection for egg production in IPB-D1 chicken. Further studies are needed to confirm this hypothesis.

ACKNOWLEDGEMENT

We thank to Ministry of Research, Technology and Higher Education (Indonesia), which has financed this research through the collaboration of World Class Research, with the contract number: 121/SPH/LT/DRPM/2019.

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Effect of *Averrhoa bilimbi* Fruit Filtrate and Shrimp Paste Mixture on Performance, Gut Microbes and Blood Profile of Broilers

Mareta I, Nathaniel G, Yudiarti T, Widiastuti E, Wahyuni HI, Sugiharto S

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java Indonesia
E-mail: sgh_undip@yahoo.co.id

(received: 30-05-2020; revised: 30-09-3030; accepted: 07-10-2020)

ABSTRAK

Mareta I, Nathaniel G, Yudiarti T, Widiastuti E, Wahyuni HI, Sugiharto S. 2020. Pengaruh kombinasi filtrat buah belimbing wuluh dan terasi terhadap performa, mikroba usus dan profil darah ayam broiler. JITV 25(4): 182-189. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2515

Penelitian bertujuan mengevaluasi pengaruh kombinasi filtrat buah belimbing wuluh (*Averrhoa bilimbi* L) dan terasi (*Mysis* sp.) terhadap performa pertumbuhan, profil darah, populasi mikroba usus dan pH saluran pencernaan ayam broiler. Campuran filtrat buah belimbing wuluh dan terasi diinkubasi selama 4 hari dan digunakan dalam penelitian. Penelitian *in vivo* menggunakan 40 ekor ayam broiler strain Lohmann umur sehari yang didistribusikan secara acak pada dua kelompok perlakuan, meliputi kontrol (T1) dan ayam yang diberikan kombinasi filtrat buah belimbing wuluh dan terasi sebanyak 10% dalam air minum (T2). Bobot badan dan konsumsi pakan dicatat setiap minggu. Pada hari ke-42, 2 ekor ayam dari setiap pen (8 ayam per kelompok perlakuan) diambil untuk pengambilan darah dan digesta. Bobot organ internal dan karkas ditimbang setelahnya. Hasil penelitian menunjukkan bahwa nilai konversi pakan (FCR) lebih rendah (P<0,05) pada ayam yang diberi kombinasi filtrat buah belimbing wuluh dan terasi dibandingkan dengan kontrol. Hemoglobin dan volume rata-rata sel darah merah (MCV) ayam perlakuan lebih tinggi (P<0,05) dari ayam kontrol. Total kolesterol darah ayam perlakuan lebih tinggi (P<0,05) dari kontrol. Total *coliform* dalam sekum ayam perlakuan lebih rendah (P<0,05) dari kontrol. Nilai pH saluran pencernaan (jejunum, ileum dan sekum) ayam yang diberikan kombinasi filtrat buah belimbing wuluh dan terasi lebih rendah (P<0,05) dari ayam kontrol. Kesimpulan pada penelitian ini bahwa pemberian campuran filtrat buah belimbing wuluh dan terasi ke dalam air minum memperbaiki FCR, meningkatkan nilai hemoglobin dan MCV, menurunkan pH usus dan bakteri *coliform* pada sekum ayam broiler.

Kata Kunci: Belimbing Wuluh, Ayam Broiler, Filtrat, Performa, Terasi

ABSTRACT

Mareta I, Nathaniel G, Yudiarti T, Widiastuti E, Wahyuni HI, Sugiharto S. 2020. Effect of *Averrhoa bilimbi* fruit filtrate and shrimp paste mixture on performance, gut microbes and blood profile of broilers. JITV 25(4): 182-189. DOI: http://dx.doi.org/10.1433/jitv.v25i4.2515

This study was aimed to evaluate effect of a mixture of *Averrhoa bilimbi* fruit filtrate and shrimp paste (*Mysis* sp.) on the growth performance, blood profile, selected intestinal bacterial number and pH value of broiler digestive tract. The mixture of *A. bilimbi* fruit filtrate and shrimp paste were incubated for 4 days and were then used in the experiment. For *in vivo* experiment, 40 day-old Lohmann broiler chicks were distributed randomly to two treatment groups, i.e., control (T1) and chickens given the mixture of 10% *A. bilimbi* fruit filtrate and shrimp paste in drinking water (T2). Body weight and feed intake were recorded weekly. At day 42, 2 birds from each pen (8 chicks per treatment group) were taken for blood and digesta collection. Internal organ weight and carcass traits were determined thereafter. Feed conversion ratio (FCR) was lower (P<0.05) on the treatment group than the control. Hemoglobin and mean corpuscular volume (MCV) of the treatment group were higher (P<0.05) than that of the control. Total cholesterol was higher (P<0.05) in the treatment group than that in control. Total cecum coliform was lower (P<0.05) in the treatment group than that in the control. The pH values of the small intestinal segments (jejunum, ileum, cecum) were lower (P<0.05) in the treatment group than that in the control group. In conclusion, administration of the blends of *A. bilimbi* fruit filtrate and shrimp paste into drinking water improved FCR, increased hemoglobin and MCV values, decreased gut pH and cecal coliform of broiler chickens.

Key Words: *Averrhoa bilimbi* L, Broiler Chicken, Filtrate, Performance, Shrimp Paste

INTRODUCTION

Antibiotic growth promoters (AGP) have long been used to boost the growth rate and control pathogenic bacteria in broiler production. However, despite its advantageous effects, the continuous use of AGP may cause microbial resistance and leave residues in the meat of broilers (Singh et al. 2014). Among the

alternatives that can be used to replace AGP in poultry production are organic acids and probiotics.

Organic acids may serve as acidifier, which may consist of citric acid, lactic acid, propionate, acetic acid or the mixture of some organic acids. Organic acid is applied as feed additive into feed or drinking water in order to enhance digestive enzyme activities, decrease the pH of the gut and maintain microbial balance in digestive tract of broilers (Octavia et al. 2018). Probiotic is a feed additive in the form of beneficial living microbes. It functions to improve ecosystem of the digestive tract of chickens (Sugiharto et al. 2017).

Organic acids may be combined with probiotics to increase the effectiveness of organic acid as an alternative to AGP (Sugiharto 2016). Abudabos et al. (2016) noticed that the combination of organic acid and *Bacillus* sp. probiotic could effectively replace AGP in terms of improving growth rate, nutrient absorption and controlling pathogenic bacteria in the gut of broilers such as *Salmonella* sp., *Escherichia coli* and *Clostridium perfringens* in the intestine of broilers.

Averrhoa bilimbi L. fruit is a sour fruit that has a potential to be used as an alternative antibiotic for broilers. *A. bilimbi* belongs to the Oxalidaceae family and is easily cultivated in tropical countries. In Indonesia, *A. bilimbi* fruit is called as *belimbing wuluh* and has traditionally been used to cure many diseases such as fever, hypertension and inflammation (Dewi et al. 2019). The fruit may also be used as a source of natural organic acids due to its high contents of acetic acid, citric acid, lactic acid, propionic acid and formic acid as well as other active substances such as phenol, vitamin C and tannin (Patil et al. 2013). Nakyinsige et al. (2016) further reported that *A. bilimbi* fruit filtrate contains antibacterial substance, which can inhibit the growth of pathogenic microbes. *A. bilimbi* filtrate also contains nutrients such as amino acid and glucose. Glucose contained in *A. bilimbi* fruit filtrate can be used by lactic acid bacteria as the source of nutrient for bacterial growth and development (Kumar et al. 2013). For this reason, *A. bilimbi* fruit filtrate has potential to be the substrates for lactic acid bacteria.

Shrimp (*Mysis* sp.) paste is a traditional food additive with special aroma and is made from the fermentation of shrimp. As with other fermented products, shrimp paste can be a natural source of lactic acid bacteria and can thus be a source of probiotics (Amalia et al. 2018). Kobayashi et al. (2003) reported that in general, the total colonies of lactic acid bacteria in Indonesian shrimp paste ranged from 4 to 6 log cfu/g. There are several lactic acid bacteria species isolated from shrimp paste, including *Lactobacillus plantarum*, *Lactococcus lactis*, *Vagococcus fluvialis* and *Lactococcus garvieae* (Maeda et al. 2014), with *L. plantarum* being the most dominant lactic acid bacteria species (Amalia et al. 2018).

Considering all facts about *A. bilimbi* fruit filtrate and shrimp paste, a mixture of *A. bilimbi* fruit filtrate and shrimp paste was therefore expected to improve the productivity and ecosystem of broiler's digestive tract. To date, publications or reports regarding the use of a mixture of *A. bilimbi* fruit filtrate and shrimp paste in broiler chicken have not been found in the literature. Therefore, this study was aimed to evaluate the effect of the mixture of *A. bilimbi* fruit filtrate and shrimp paste on growth performance, blood profile, selected intestinal bacterial number and pH value of broiler digestive tract.

MATERIALS AND METHODS

Preparation of the mixture of *A. bilimbi* fruit filtrate and shrimp paste

In this study, ripe (yellowish peel and soft texture) of *A. bilimbi* and shrimp paste which obtained from the district of Rembang were used. *A. bilimbi* fruit was crushed using electric blender and filtrated using cheesecloth. The filtrate of *A. bilimbi* fruit was placed into an anaerobe jar, and shrimp paste was then added (1 g/1000 ml) into it. The mixture of *A. bilimbi* fruit filtrate and shrimp paste was incubated for 4 days at room temperature in anaerobic conditions. After the incubation, the sample pH was determined and enumerated for lactic acid bacteria content. The preparation protocol was based on our preliminary *in vitro* study for assessing the best ripening stages of *A. bilimbi* fruit, concentration and brands of shrimp paste and time and condition of incubation (data published elsewhere). The number of lactic acid bacteria was determined based on total plate count method on *de Man, Rogosa* and *Sharpe agar* media (MRS; Merck KGaA, Darmstadt, Germany). The enumeration of bacteria was conducted after the sample was incubated in an anaerobic condition at 38°C for 48 hours. The pH value of the unincubated *A. bilimbi* fruit filtrate and after incubation with shrimp paste for 4 days were 1.5 and 1.2, whereas the number of lactic acid bacteria of the unincubated *A. bilimbi* fruit filtrate and after incubation with shrimp paste for 4 days were 5.30 log cfu/mL, and 30.56 log cfu/mL, respectively. The mixture was prepared for *in vivo* study and stored at -10ºC until used.

In vivo experiment and performance analysis

The *in vivo* experiment complied with the standard broiler rearing protocols established by the Laboratory of Poultry Production, Faculty of Animal and Agricultural Sciences, Diponegoro University. The trial was carried out on February to March 2020. The

experiment was conducted on 40 day-old Lohmann broiler chicks with the average initial body weight of 47.3±0.78 g. During the rearing period, the chicks were fed with starter, grower and finisher rations. The mixture of *A. bilimbi* fruit filtrate and shrimp paste was added into drinking water as much as 10%. The feeds (in mash form) and drinking water were provided *ad libitum* throughout the study period. The chickens were vaccinated with Newcastle disease (ND) vaccine at 4 days of age through eye drop, IBD/Gumboro vaccine at 10 days of age and ND vaccine at the age of 18 days, both through drinking water. In this study, the chicks were divided into 2 treatment groups with 4 pens consisting of 5 chickens in each group. These groups were T1 (control, i.e., chickens given drinking water without the mixture) and T2 (chickens given drinking water containing 10% of the mixture).

During *in vivo* trial, the birds were raised in an open-sided broiler house with rice husk as bedding materials. They (5 chicks) were placed in 1×1 m^2 pen equipped with manual feeder and water container. The temperature and humidity inside the broiler house were adjusted using plastic curtains and electric fan. Temperature was adjusted at 32±1ºC on initial brooding period and then gradually reduced according to the age of broilers (around 27±1ºC on day 21 onward).

Body weight and feed consumption were recorded at weekly basis. At the end of experiment (day 42), 2 chickens from each pen (8 chicks per treatment group) were taken for sample collection. The chickens were weighed and then their blood was withdrawn through brachialis veins using 3 mL syringe. One mL blood was put into a tube given anticoagulants (ethylene diamine tetra acetic acid, EDTA) while the other 2 mL blood was put into a tube without anticoagulants. The non-anticoagulant blood was then centrifuged at 5,000 rpm for 15 minutes to obtain the serum. After blood sampling, the chicken was slaughtered and the digestive tract was taken. The digesta in the small intestine and ceca was collected. Some of the digesta was put into zip lock plastic bag for the determinations of selective bacteria enumerations. The rest of the intestinal digesta was used for pH measurement. The weight of internal organ, carcass and commercial cuts were also determined.

Blood profile analysis

Complete blood analysis was conducted using hematology analyzer (Prima Fully-auto Hematology Analyzer) and total counts of bacteria in the intestinal digesta were performed according to Sugiharto et al. (2018). Total triglycerides in serum was measured using enzyme calorimetric method with glycerol-3-phosphateoxidase (GPO). The triglycerides were determined after enzymatic separation using lipoprotein lipase, whereas total cholesterol was determined after enzymatic hydrolysis and oxidation. Both used indicator of quinoneimine produced from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase (DiaSys Diagnostic System GmbH, Holzheim, Germany). The LDL was precipitated using heparin. The HDL remained in the supernatant after centrifugation and was enzymatically processed by the CHOD-PAP method. The LDL concentration was calculated as the difference between total cholesterol and cholesterol in the supernatant. Coliform bacteria were determined in MacConkey agar (Merck KGaA) as red colonies after aerobic incubation at the temperature of 38°C for 24 hours. The number of lactic acid bacteria was enumerated on MRS (Merck KGaA) after anaerobic incubation at the temperature of 38°C for 48 hours.

Statistical analysis

Data were analyzed using independent-samples t-test with 5% accuracy rate (Sudjana 1989). The analysis was conducted using SPSS 22.0 software.

RESULTS AND DISCUSSION

Performances of broilers

Results of the present study show that addition of the mixture of *A. bilimbi* fruit filtrate and shrimp paste at 10% to drinking water decreased ($P<0.05$) FCR of broilers (Table 1). Nevertheless, the treatments did not significantly affect the body weight gain (BWG) and feed consumption of broilers. The improvement of FCR in the treated birds was possible because the administration of the mixture, as source of organic acid and probiotic improves the digestive process particularly protein through the increase in digestive enzyme activities (Salgado-Tránsito et al. 2011). Marín-Flamand et al. (2013) suggested that administration of organic acid mixtures of ascorbic, citric, malic, sorbic and tartaric acids would trigger the secretion of hormones such as gastrin and cytokinin, which will improve the digestion and absorption of feed protein, so that FCR of broilers would consequently improve. Likewise, Jin et al. (2000) reported that supplementation of *Lactobacillus* culture increased digestive enzyme activities, which thereby improved the digestion and utilization of nutrients by broilers.

Table 1. Performances of broilers

Items	T1	T2	SEM	P value
Body weight gain (g/bird)	1,839	1,913	70.0	0.64
Feed consumption (g/bird)	3,762	3,374	107	0.08
Feed conversion ratio (FCR)	2.05	1.76	0.06	<0.01

SEM: standard error of the means

Table 2. Blood profile of broilers

Items	T1	T2	SEM	P value
Leukocyte (10^9/L)	78.6	74.5	2.11	0.36
Erythrocyte (10^{12}/L)	2.84	3.32	0.13	0.07
Hemoglobin (g/dL)	9.25	12.0	0.54	0.01
Hematocrit (%)	37.8	41.4	1.42	0.21
Thrombocyte (10^9/L)	8.87	9.75	0.42	0.32
Lymphocyte (%)	67.3	76.8	3.03	0.12
Neutrophil (10^9/L)	3.56	4.37	0.52	0.46
Mean corpuscular volume (fl)	116	127	1.97	<0.01
Mean corpuscular hemoglobin (pg)	31.5	33.7	0.71	0.12
Mean corpuscular hemoglobin concentration (g/dL)	25.3	27.6	0.61	0.06

SEM: standard error of the means

Table 3. Lipid profile in serum of broilers

Items (mg/dL)	T1	T2	SEM	P value
Total cholesterol	96.4	120	5.68	0.04
Low-density lipoprotein (LDL)	26.7	36.1	4.11	0.27
High-density lipoprotein (HDL)	59.0	68.6	4.37	0.28
Total triglycerides	53.3	63.7	4.67	0.28

SEM: standard error of the means

Blood profile of broilers

Results showed that addition of the mixture into drinking water increased (P<0.05) hemoglobin and MCV values of broilers (Table 2). However, the treatments did not affect (P>0.05) the numbers of leukocytes, erythrocyte, hematocrit, thrombocyte, lymphocyte, neutrophil, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). In this study, the increased values of hemoglobin and MCV seemed to be attributed to the antioxidant components in the mixture in the form of saponin, tannin, flavonoids, terpenoids and steroids, which can prevent oxidative stress and improve blood profile of broiler chickens (Asna & Noriham 2014) Surai (2016) suggested that antioxidants possess the ability to minimalize free radical as well as to prevent the negative effect of free radicals by completing electron lacking in cell which is caused by exposure to free radicals.

The addition of electrons by antioxidant compound in cells can prevent the damage of nucleic acid, protein, fat and deoxyribonucleic acid (DNA). It has been understood that amino acids are the main components necessary for the process of blood synthesis. This means that the more nutrient components such amino acids and protein are available, the more optimum the process of blood synthesis. Optimum process of hematopoiesis will improve the blood profiles (Adil et al. 2011). Hemoglobin plays an important role in loading and binding oxygen in the lung and releasing it to parts of the body which need it. Considering that oxygen is needed in the process of metabolism, high total of hemoglobin and mean corpuscular volume

(MCV) will therefore make the metabolic process more optimum in broilers (Ugwuene 2011).

Lipid profile of broilers

The result showed that administration of the mixture into drinking water increased (P<0.05) total cholesterol in the serum of broilers (Table 3). However, the treatments did not influence (P>0.05) the levels of LDL, HDL and triglycerides in the serum. In this work, the increase in serum total cholesterol was in parallel with the increased LDL, HDL and total triglycerides values in the serum, though the differences were not statistically significant. Formerly, Ndelekwute et al. (2016) documented that organic acids administration was associated with the enhanced fat digestibility and utilization by broilers.

Considering the positive correlation between fat utilization (and metabolism) and blood cholesterol levels (Saleh et al. 2020), the possible increased fat digestibility and utilization in broilers treated with the mixture of *A. bilimbi* fruit filtrate and shrimp paste may therefore be attributed to the increased total serum cholesterol in the respective chicks. However, the latter inference should be noted with caution as we did not conduct the digestibility trial in this present study. Also, our finding was different from that of Taherpour et al. (2009) who reported that combination of organic acid and probiotic decreased serum total cholesterol in broiler chickens. Other study by Yakhkeshi et al. (2011) also reported that the use of probiotic as feed additive in broilers ration decreased total cholesterol of broilers. The differences in the natures and levels of organic acids as well as lactic acid bacteria between our product and the products used by other investigators were most likely be responsible for the divergent results above.

pH values and bacterial populations of intestine of broilers

The present study showed that addition of the mixture into drinking water decreased (P<0.05) pH values of jejunum, ileum and cecum (Table 4), but did not significantly affect pH value of duodenum of broilers. Sugiharto (2016) documented that the use of organic acid separately or in combination with probiotic decreased the pH values of the entire intestinal segments and may function as antibacterial substance to inhibit the growth of pathogenic microbes in the digestive tract of broilers. *A. bilimbi* fruit filtrate as the source of organic acids and shrimp paste as the source of lactic acid bacteria-based probiotic may promote the production of lactic acid and other short chain fatty acids (SCFA; acetic, butyric and propionic acids), which are generally known to decrease the pH values of the digestive tract of broilers (Dittoe et al. 2018).

The results of this study showed that addition of the mixture into drinking water decreased (P<0.05) total coliform in the cecum of broilers (Table 4). However, the treatment had no significant effect on coliform count in ileum and lactic acid bacteria populations in ileum and cecum of broilers. Nakyinsige et al. (2016) reported that *A. bilimbi* fruit filtrate contained some bioactive compounds including phenol and tannin, which can inhibit the growth of pathogenic microbes in the digestive tract of broilers. The latter study was confirmed by Tosi et al. (2013) documenting that tannin can function as antibacterial substance by coagulating bacteria protoplasm so that pathogenic bacteria cannot replicate and finally lysis. Sugiharto (2016) reported that lactic acid bacteria-based probiotic can perform competitive exclusion, which is a competition between pathogenic microbes such as coliform and non-pathogenic microbe such as lactic acid bacteria. Both types of bacteria compete to obtain nutrition and reside in small intestine of broilers. Moreover, lactic acid bacteria were able to produce lactic acid, which can inhibit the growth of pathogenic microbes in the digestive tract of broilers. Furthermore, Sumarsih et al. (2012) reported that lactic acid bacteria may produce natural antibiotic compound, which able to kill pathogenic microorganisms in broiler digestive tract.

Internal organ weights and carcass characteristics of broilers

Results of the experiment showed that administration of the mixture into drinking water did not affect (P>0.05) internal organ relative to weights of broilers (Table 5). This finding was in accordance with Youssef et al. (2017) confirming that administration of the combination of organic acid and probiotic into ration did not affect the relative weights of internal organ of broilers. In concurrence with this, Sugiharto et al. (2018) showed no impact of dietary administration of multi-strains probiotic combined with vitamins and minerals on the relative weights of internal organs of broilers.

The results showed that addition of the mixture into drinking water did not affect (P>0.05) the carcass weight and commercial cuts of broilers (Table 5). Malik et al. (2016) also reported that supplementation of the combination of organic acid and probiotic into rations did not make significant difference in carcass weight and commercial cuts of broilers. In general, some factors may affect the carcass characteristics of broilers, including genetic, sex, physiological status, age, final live body weight and nutrients (Hidayat et al. 2017).

Table 4. pH values and intestinal bacterial populations of intestine of broilers

Items	T1	T2	SEM	P value
pH values				
Duodenum	5.82	5.45	0.12	0.13
Jejunum	5.70	4.77	0.18	0.01
Ileum	6.00	4.95	0.20	<0.01
Cecum	6.54	6.07	0.10	0.02
Bacterial populations				
Coliform in ileum	5.50	5.06	0.28	0.20
Coliform in cecum	5.37	4.55	0.15	<0.01
Lactic acid bacteria in ileum	8.19	8.54	0.06	0.09
Lactic acid bacteria in cecum	8.70	8.85	0.27	0.06

SEM: standard error of the means

Table 5. Internal organ weights and carcass traits of broilers

Items	T1	T2	SEM	P value
Internal organ weights (% live body weight)				
Heart	0.33	0.31	0.01	0.40
Liver	1.72	1.63	0.08	0.63
Proventriculus	0.41	0.36	0.01	0.11
Gizzard	1.63	1.58	0.09	0.78
Pancreas	0.18	0.17	0.01	0.80
Abdominal fat	1.03	1.30	0.09	0.19
Duodenum	0.26	0.33	0.02	0.15
Jejunum	0.67	0.70	0.04	0.71
Ileum	0.49	0.58	0.03	0.15
Cecum	0.43	0.35	0.05	0.45
Spleen	0.09	0.08	0.01	0.35
Thymus	0.11	0.10	0.01	0.63
Bursa of Fabricius	0.03	0.03	0.00	0.25
Carcass traits				
Carcass weight (% live body weight)	54.2	54.8	1.55	0.87
Breast (% carcass)	35.8	35.2	0.51	0.56
Wings (% carcass)	11.6	11.3	0.24	0.42
Thigh (% carcass)	16.9	17.1	0.29	0.74
Drumstick (% carcass)	14.6	14.7	0.28	0.81
Back (% carcass)	21.1	21.7	0.54	0.56

SEM: standard error of the means

CONCLUSION

Administration of the mixture of *A. bilimbi* fruit filtrate and shrimp paste into drinking water improved FCR, increased hemoglobin and MCV values, decreased the pH of the gut and the number of faecal coliform of broiler chickens. In conclusion, the mixture was beneficial in improving feed efficiency, physiological condition and intestinal ecology of broiler chickens which inhibited the growth of pathogenic microbes in the digestive tract of broilers.

ACKNOWLEDGEMENT

The study was financially supported by Directorate of Research and Community Service, Directorate General of Higher Education, Ministry of Education and Culture, Republic of Indonesia (Contract No. 225-88/UN7.6.1/PP/2020, 20 March 2020).

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Chemical Quality of Culled Duck Meatball (*Anasplathyryncos*) Substituted with Edamame Flour (*Glycine max* (L) Merril) Filler

Prayitno AH, Rahman TH

Department of Animal Science, Politeknik Negeri Jember
Jl. Mastrip Po Box 164 Jember - Indonesia
E-mail: agushp@polije.ac.id

(received 30-05-2020; revised 03-07-2020; accepted 26-09-2020)

ABSTRAK

Prayitno, AH and Rahman, TH. 2020. Kualitas kimia bakso daging itik afkir (*Anasplathyryncos*) yang disubstitusi *filler* tepung edamame (*Glycine max (L) Merril*). JITV 25(4): 190-194. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2514

Penelitian ini bertujuan untuk mengetahui pengaruh subtitusi filler tepung edamame terhadap kualitas kimia bakso daging itik afkir. Materi penelitian terdiri dari daging itik afkir, tepung tapioka, tepung edamame, putih telur, bawang putih, bawang merah, garam, lada, monosodium glutamat, sodium tripolifosfat, dan es. Perlakuan substitusi filler tepung edamame yaitu P0 (0%), P1 (5%), P2 (10%), P3 (15%), dan P4 (20%) dari total filler. Setiap perlakuan terdiri dari lima replikasi. Parameter yang diuji yaitu kadar air, protein, lemak, serat, dan abu. Data hasil uji kualitas kimia dianalisis dengan analisis variansi rancangan acak lengkap pola searah dan jika terdapat perbedaan yang signifikan (P<0,01) kemudian diuji lanjut dengan uji Duncan's New Multiple Range Test. Hasil penelitian menunjukan bahwa substitusi filler tepung edamame mulai dari level 5% sudah sangat berpengaruh terhadap kadar air, protein, lemak, serat, dan abu bakso daging itik afkir. Tepung edamame dapat dijadikan sebagai substitusi filler sampai level 20% dengan memberi pengaruh baik terhadap peningkatan kandungan protein bakso, tetapi juga berdampak negatif dengan meningkatnya kandungan lemak bakso daging itik afkir.

Kata Kunci: Bakso, Daging Itik Afkir, *Filler*, Kualitas Kimia, Tepung Edamame

ABSTRACT

Prayitno, AH and Rahman, TH. 2020. Chemical quality of culled duck meatball (*Anas plathyryncos*) substituted with edamame flour (*Glycine max* (L) Merril) filler. JITV 25(4): 190-194. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2514

This study aimed to determine the effect of edamame flour filler substitution on the chemical quality of culled duck meatballs. The research material consisted of culled duck meat, tapioca flour, edamame flour, albumen, garlic, onion, salt, pepper, monosodium glutamate, sodium tripolyphosphate, and ice. The edamame flour filler substitution treatments were P0 (0%), P1 (5%), P2 (10%), P3 (15%), and P4 (20%) from total filler. Each treatment consisted of five replications. The parameters which tested were moisture, protein, fat, fiber, and ash contents. Data on chemical quality results were analyzed by analysis of variance using completely randomized design and if there was significantly different (P<0.01), then it'll be further tested by the Duncan's New Multiple Range Test. Results showed that the substitution of edamame flour filler starting from the level of 5% was significantly affected water content, protein, fat, fiber, and ash of culled duck meatballs. Edamame flour can be used as a filler substitution up to 20% level by giving a good effect on increasing the meatball protein content, but also having a negative impact with increasing the fat content of culled duck meatballs.

Key Words: Meatball, Culled Duck Meat, Filler, Chemical Quality, Edamame Flour

INTRODUCTION

Ducks were one type of waterfowl that the meat was less desirable to the community because it has an off-odor (Anggraini et al. 2017), rough textured and tough so low meat quality (Hafid et al. 2015; Smith et al. 2015). The volatile components derived from the oxidation of unsaturated fats were the cause of duck meat has an off-odor (Purba et al. 2010).This meat has a higher fat, protein content and lower calories rather than other poultry meats (Utami et al. 2011). The color of duck meat was darker than chicken meat (Huda et al. 2011). The chemical compositions of duck meat were 73.29-80.69% moisture, 19.99-24.34% protein, 1.05-1.18% ash (Qiao et al. 2017), 1.4% carbohydrate (Biswas et al. 2019), 1.55-2.30% intramuscular fat (He et al. 2018), and 12.21-28.21% fat (Lestari et al. 2015).

Quality of culled duck meat can be improved by processing as meatballs. Processing of duck meat into meatballs can reduce off-odor from duck meat (Anggraini et al. 2017). Duck meat processed into meatballs was preferred than other processed products (Putra et al. 2011; Kusmayadi & Sundari 2019). The meatballs were one of the processed meat products made by grinding the meat, mixed with flour and spices, then formed into balls and boiled until cooked

with hot water (Chakim et al. 2013). Meatballs are usually made from beef (Malini et al. 2016).

Increasing number of Indonesian population who are so busy resulted in the pattern of consumption of ready to cook meat and eat has developed so rapidly (Prayitno et al. 2019) one of which was meatballs, that have high acceptability and nutritional value (Prayitno et al. 2016; Prayitno et al. 2019). Chemical compositions of meatball consist of 8% minimum protein, 10% maximum fat, 70% maximum moisture, and 3% maximum ash (SNI 2014). Meatballs can be produced using duck meat (Nurkhoeriyati et al. 2012; Murti et al. 2013; Haslia et al. 2015; Lestarini et al. 2015; Biswas et al. 2019).

Meatballs as emulsion products are usually contain fillers in the form of tapioca flour. Tapioca flour in the processing of culled duck meatballs can be substituted with edamame flour as filler. The filler substitution in processed meat products as an innovation to optimize local resources (Syam et al. 2019). Edamame has been used as filler in the processing of sausages. Edamame was a Japanese variety of green soybeans and large pods (Suryaningsih 2013) and contains bioactive components (Aliyah & Setiawati 2018; Widiyawati & Susindra 2018). Edamame production in Indonesia was widely developed in Jember. Chemical compositions of edamame flour consist of 3.22% moisture, 40.02% protein, 18.43% fat, 34.65% carbohydrate, and 3.78% ash. Edamame flour based on its chemical compositions can be used as a filler and binder with high protein and carbohydrate contents. The chemical quality of meatballs was one of the important parameters in determining nutritional value of meatballs. This study aimed to determine the effect of edamame flour filler substitution on the chemical quality of the culled duck meatballs. Results of this study were expected to become information about the use of edamame flour as filler in the processing of culled duck meatballs.

MATERIALS AND METHODS

Edamame flour processing

Edamame skin was peeled. Edamame seeds were crushed, and then dried at 60°C for 24 hours and then ground until smooth then sieved using a filter with a size of 60 mesh. Filtered edamame flour was used as filler for the processing of culled duck meatballs. The scheme of edamame flour processing was presented at Figure 1.

Meatball processing

The culled duck meatball formulation in this study was made based on a modification of Prayitno et al. (2019). The boneless duck meat was cut into small pieces and then ground using meat grinder, followed by the addition of salt, pepper, monosodium glutamate (MSG), sodium tripolyphosphate (STPP), garlic, onion, albumen, tapioca flour, edamame flour according to treatment, and ice then ground until all mixed. The meatball dough was formed balls boiled in boiling water for 10 minutes then cooled for 15 minutes. The

Table 1. The formulation of culled duck meatballs substituted with edamame flour filler

Ingredients	Treatments				
	P0	P1	P2	P3	P4
Culled duck meat (%)	60	60	60	60	60
Tapioca flour (%)	15	14.25	13.5	12.75	12
Edamame flour (%)	0	3.75	7,5	11.25	15
Albumen (%)	10	10	10	10	10
Garlic (%)	2.5	2.5	2.5	2.5	2.5
Onion (%)	1	1	1	1	1
Salt (%)	1.5	1.5	1.5	1.5	1.5
Pepper (%)	1	1	1	1	1
Monosodium glutamate (%)	1	1	1	1	1
Sodium tripolyphosphate (%)	1	1	1	1	1
Ice (%)	7	7	7	7	7
Total (%)	100	100	100	100	100

P0 (0%), P1 (5%), P2 (10%), P3 (15%), and P4 (20%) substitution edamame flour from total filler

cooked meatballs then tested on chemical quality. The scheme of culled duck meatball processing was presented at Figure 2. The formulation of substitution with edamame flour filler was presented at Table 1.

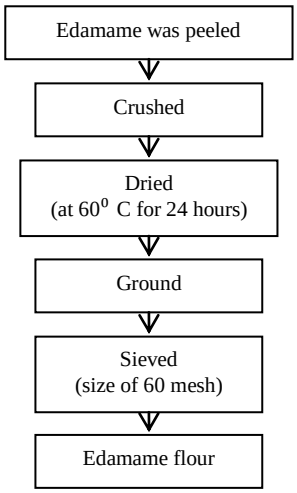

Figure 1. The scheme of edamame flour processing

Chemical quality analysis

The chemical composition of culled duck meatballs substituted with edamame flour filleranalyzed following standard AOAC methods (AOAC 2019). The proximate measurements were moisture, protein, fat, fiber, and ash contents.

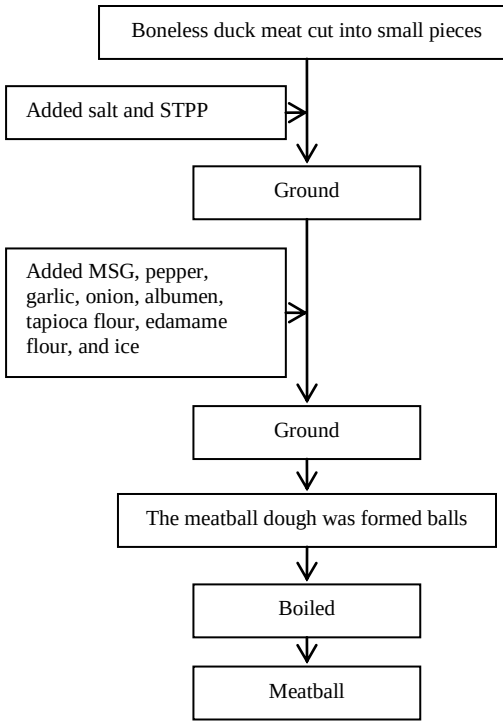

Figure 2. The scheme of meatball processing

Statistical analysis

The data were analyzed by analysis of variance using completely randomized design and if there was significantly different (P<0.01), then tested further by the Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Chemical quality of meatballs was one of the important parameters that become the standard in determining the nutritional value of meatballs. The chemical quality was presented in Table 2.

Moisture content

Results showed that the culled duck meatball substituted with edamame flour filler had a highly significant effect (P<0.01) on the moisture content of the meatball. The moisture content ranged from 45.05-46.93%. This result was lower than that of beef meatball of 73.70-74.61% (Malini et al. 2016). Edamame flour substitutions were increased from 5% to 20%, followed by significantly decreased in water content of the meatballs. This was because edamame flour has lower water content than tapioca flour. Edamame flour has a moisture content of 3.22%, while tapioca flour has a moisture content of 8.36% (Safitri et al. 2017). The moisture content from his study was in agreement with the standard because it was below the maximum limit of the meatball moisture content of 70% (SNI 2014). This result was lower than that resulted by Anggraini et al. (2017), the meatball water content was 65.40-67.26%.

Protein content

Results showed that the substitution of filler had a highly significant effect (P<0.01) on the protein content of the meatball. The meatball protein levels ranged from 10.61-16.13%. The protein content of this meatball was higher than the protein content of beef meatball of 11.22-12.10% (Malini et al. 2016). As the substitutions of filler increased from 5% to 20%, protein content of the meatballs increased significantly. This was because edamame flour has higher protein content than tapioca flour. Edamame flour has a protein content of 40.02%, while tapioca flour has a protein content of 3.05% (Safitri et al. 2017). The protein content of the meatball was in agreement with the standard because it was above the minimum limit of the meatball protein content of 8% (SNI 2014). This result was higher than that resulted by Nurkhoeriyati et al. (2012), the meatball protein was 6.58-7.67%.

Table 2. The chemical composition of culled duck meatballs substituted with edamame flour filler

Variables	Treatments				
	P0	P1	P2	P3	P4
Moisture (%)	46.93[a]	46.43[b]	46.09[c]	45.58[d]	45.05[e]
Protein (%)	10.61[a]	11.68[b]	13.11[c]	14.60[d]	16.13[e]
Fat (%)	17.16[a]	17.81[b]	18.51[c]	19.18[d]	19.86[e]
Fiber (%)	0.11[a]	0.57[b]	1.11[c]	1.55[d]	2.24[e]
Ash (%)	0.84[a]	0.93[b]	1.03[c]	1.14[d]	1.19[e]

[abcde] Means in the same row with different lettersuperscriptswere significantly different (P< 0.01)

Fat content

Results showed that the culled duck meatball substituted with edamame flour filler had a highly significant effect (P<0.01) on the fat content of the meatball. The fat content ranged from 17.16-19.86%. The fat content from this study was higher than the fat content of beef meatball of 1.59-2.27 % (Malini et al. 2016). As Edamame flour substitutions increased from 5% to 20%, the fat content significantly increased. This was because edamame flour has higher fat content than tapioca flour. Edamame flour has a fat content of 18.43%, while tapioca flour has a fat content of 0.12% (Safitri et al. 2017). The fat content exceeded the standard because it was above the maximum limit of the meatball fat content of 10% (SNI 2014). This result was higher than that of Lestari et al (2015), namely 12.21-28.21%. Consuming high-fat content foods might increase obesity and risk of degenerative diseases (Freeman et al. 2014).

Fiber content

The substitution of edamame flour filler had a highly significant effect (P <0.01) on the level of meatball fiber. The meatball fiber levels ranged from 0.11-2.24%. This result was almost the same as the beef meatball fiber content of 0.13-4.86% (Hu & Yu 2015). The increased edamame flour substitutions from 5% to 20%, followed by significantly increased in fiber content of the meatballs. This was because edamame flour has higher fiber content than tapioca flour. Edamame flour has a fiber content of 3.27% (Widiyawati& Susindra 2018), while tapioca flour has a fiber content of 2.18% (Charoenkul et al. 2011). The levels of culled duck meatball substituted with edamame flour filler were following previous studies which stated that the levels of meatball fiber ranged from 0.26-2.90% (Kurniawan et al. 2012).

Ash content

The substitution of edamame flour filler had a very significant effect (P<0.01) on ash content of the meatball. It was ranged from 0.84-1.19%. The ash content of culled duck meatball was higher than the ash content of beef meatball of 2.28- 2.35% (Malini et al. 2016). As the substitution increased from 5% to 20%, the ash content significantly increased. This was because edamame flour has higher ash content than tapioca flour. Edamame flour has a protein content of 3.78%, while tapioca flour has a protein content of 2.39% (Safitri et al. 2017). The content of duck meatball ash substituted with edamame flour filler is in accordance with the standard because it is below the maximum limit of the meatball ash content of 3% (SNI 2014).

CONCLUSION

The results showed that the substitution of edamame flour filler starting from the level of 5% was significantly affect water content, protein, fat, fiber, and ash of culled duck meatballs. Edamame flour can be used as a filler substitution up to 20% level by giving a good effect on increasing the meatball protein content, but also might have negative impact with increasing the fat content of the meatballs.

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Effects of Probiotic, Prebiotic, and Synbiotic Mixed Culture Based on Wheat Pollard on Productivity of Kampung's Chicken

Utama CS[1a], Zuprizal[2], Hanim C[2], Wihandoyo[2]

[1]*Feed Technology Laboratory, Department of Animal Sciences, Faculty of Animal and Agricultural Sciences, Diponegoro University*
[2]*Faculty of Animal Science, Gajah Mada University, Yogyakarta*
Jl. Fauna No. 3 Bulaksumur, Yogyakarta 55281- Indonesia
E-mail[a]: cahyasetyautama@gmail.com

(received 23-04-2020; revised 24-09-2020; accepted 26-09-2020)

ABSTRAK

Utama CS, Zuprizal, Hanim C, Wihandoyo. 2020. Pengaruh pemberian probiotik, prebiotik dan sinbiotik *mixed culture* berbasis *wheat pollard* terhadap produktivitas ayam kampung. JITV 25(4): 195-205. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2499

Penelitian bertujuan menguji efektivitas ransum berbasis *wheat pollard* terolah untuk meningkatkan produktivitas ayam kampung sampai umur 8 minggu. Penelitian menggunakan rancangan acak lengkap pola searah dengan lima perlakuan dan empat ulangan. Perlakuan terdiri dari ransum berbasis *wheat pollard* (WP), ransum berbasis *wheat pollard* plus probiotik *mixed culture (*WPPro), ransum berbasis *wheat pollard* prebiotik *mixed culture* (WPPre), ransum berbasis *wheat pollard* sinbiotik *mixed culture* 40% (WPS40) dan ransum berbasis *wheat pollard* sinbiotik *mixed culture* 60% (WPS60). Parameter yang diamati adalah konsumsi ransum, bobot badan akhir, konversi pakan (FCR), pertambahan bobot badan, retensi nitrogen, *income over feed chick cost* (IOFCC) dan profil vili usus. Hasil penelitian memperlihatkan bahwa bobot badan akhir, pertambahan bobot badan, retensi nitrogen, IOFCC dan profil vili usus halus (duodenum, jejenum, ileum) nyata (P<0,05) dipengaruhi oleh perlakuan. Penambahan sinbiotik *mixed culture* yang dibuat dari *wheat pollard* sebanyak 40% (WPS 40) dalam ransum mampu meningkatkan produktivitas ayam kampung sampai umur 8 minggu.

Kata Kunci: *Wheat Pollard*, Ayam Kampung, Sinbiotik *Mixed Culture*

ABSTRACT

Utama CS, Zuprizal, Hanim C, Wihandoyo. 2020. Effects of probiotic, prebiotic, and synbiotic mixed culture based on wheat pollard on productivity of Kampung's chicken. JITV 25(4): 195-205. DOI: http://dx.doi.org/10.14334/jitv.v25i4.2499

This research was aimed to assess the effectiveness of processed wheat pollard -based rations to increase the productivity of Kampung chickens raised until 8 weeks old. The research was carried out in a completely randomized design with 5 treatments and 4 replications. The treatments consisted of wheat pollard based ration (WP), wheat pollard based ration plus probiotic mixed culture (WPPro), wheat Pollard as prebiotic mixed (WPPre), wheat pollard as synbiotic mixed culture ration 40% (WPS40), wheat pollard as synbiotic mixed culture ration 60% (WPS60). The parameters observed were feed consumption, final body weight, feed conversion ratio (FCR), weight gain, nitrogen retention, income over feed and chick cost (IOFCC) and profiles of intestinal villus. Results showed a significant effect of the treatments on the final body weight, weight gain, nitrogen retention, IOFCC and profiles of small intestinal villus (duodenum, jejenum, ileum). It was concluded that the inclusion of 40% wheat pollard synbiotic mixed culture (WPS 40) in the ration was able to increase the productivity of kampung chickens reared until 8 weeks old.

Key Words: Wheat Pollard, Kampung Chicken, Synbiotic Mixed Culture

INTRODUCTION

Kampung chicken is one of the native chickens of Indonesian. Various methods are used to improve the productivity of kampung chickens, one of which is the utilization of quality feed. The feed contained 20% protein and 2800 kcal/kg of metabolic energy produced the highest body weight and ration efficiency in kampung chicken (Permadi et al. 2020). Resnawati & Bintang (2014) stated that the ingesta in the crop of 6 weeks old kampung chicken could be used as a reference to calculate the needs of fiber, fat, calcium, and phosphorus. In the feed formulation that consisted of local feed ingredients, the addition of functional feed to improve the performance of the chickens may be needed. The functional feed is a feed that contains active ingredients other than nutrients. One of them is a mixed culture of synbiotic feed. Synbiotic mixed culture is created from probiotic and prebiotic. The use of synbiotics is more efficient than a single use of probiotics or prebiotics separately (Gourbeyre et al. 2011).

Utama & Setiani (2014) stated that synbiotic is a fermented product derived from the enhancement in ability of probiotic bacteria due to the availability of specific substrates (prebiotics) for fermentation. The addition of synbiotic in rations was very effective in promoting growth, endurance, and beneficial microflora composition in poultry (Alloui et al. 2013; Mookiah et al. 2014). Hartono et al. (2016) stated that the addition of synbiotics influenced the condition of intestinal microflora, it increased the number of lactic acid bacteria, decreased the number of *Escherichia colli*, increased the height and width of villi. The addition of synbiotics could improve intestinal performance so that the absorption of nutrients is more optimal (Solis de los Santos et al. 2005). The addition of synbiotics to chickens rations could affect the histology of the duodenum caused by the enhancement of beneficial bacterial populations and stimulates vascularization and the development of villi. Synbiotics increased the thickness of the intestine, increased the nutrients absorbed, and reduced the metabolic needs of the digestive system (Iannitti & Palmieri 2010).

Intestinal histomorphology indicates the health status and productivity of livestock. Increase villi length, villi width, crypt depth, and crypt width in the intestine are indicated by livestock productivity and health status (Hidayat et al. 2016). The provision of multi-strain probiotics can improve immunity and control pathogenic bacteria in native chickens. Short-chain fatty acid (SCFA) produced by probiotics play a role in the multiplication of intestinal epithelial cells (Sugiharto et al. 2018). SCFA in particular, butyrate is a component of cell membrane phospholipids produced by *Bifidobacteria and Lactobacilli* through anaerobic fermentation processes in the intestine (den Besten et al. 2013). Intestinal villi profile strongly influenced the feed flow rate so it will affect the absorption of feed nutrients, epithelial, and enterocyte cell production (Perić et al. 2010; Abdel-Raheem et al. 2012; Gómez et al. 2012).

The innovation of this research was processing waste into additives that functioning as probiotics, prebiotics, and synbiotic mixed culture with fermented cabbage as the source of mixed culture probiotic and wheat pollard as a source of mixed prebiotics. Besides this research provides information on methods or ways of making additives that are easy and inexpensive through the application of fermentation technology, so it can be applied by farmers easily. The mixed cultures in this study were a combination of probiotic microorganisms from fermented cabbage (*Lactobacillus brevis*, *Lactobacillus plantarum*, *Rhizopus oryzae* and *Saccharomyces cerevisiae*) as well as a combination of food fiber such as arabinosa, mannosa, raffinosa, and starch resistant which are contained in wheat pollard processed as prebiotic. This study was done to assess the effectiveness of wheat pollard-based rations to improve the productivity of native chicken until the age of 8 weeks.

MATERIALS AND METHODS

The research material consisted of 200 day old chicks (DOC) of kampung chicken, where 160 were allocated for wheat pollard study, and the rest of 40 chicks were used for nitrogen retention study. The average initial weight of kampung chicken was 38.0 g. The DOCs were obtained from the Maron chicken Satker (Government Unit under the Regency of Livestock Services) of Temanggung Regency. The feed was formulated consisted of ground corn, wheat pollard, prebiotic mixed, mixed culture synbiotic, soybean meal, mixed culture probiotics from fermented cabbage, mineral-vitamin mix, L-lysin HCl and DL-methionine.

The chemicals used were HCl 0.2 N, formalin, neutral buffer 10% formalin (BNF), and disinfectant. The equipment used is fermenters, litter cages for raising native chickens equipped with feed and drinking containers, battery cages, brooders for chicken warmers, sprayers, controlled dryers, autoclaves, disc mills, digital scales with an accuracy of 0.1 g and microscopes.

Methods

The research was carried out in a completely randomized design with 5 treatments and 4 replications. Each unit of replication consisted of 8 DOC. The treatments were applied for 8 weeks with the combination as: WP (Wheat pollard based ration), WPPro (Wheat pollard based ration plus probiotic mixed culture), WPPre (Wheat Pollard as prebiotic mixed), WPS40 (Wheat pollard as synbiotic mixed culture ration 40%), WPS60 (Wheat pollard as synbiotic mixed culture ration 60%)

Research procedure

The research was started by producing additives (probiotic mixed culture, prebiotic mixed, and synbiotic mixed culture). All dietary treatments were adjusted to meet the nutrient requirements of the chickens.

Production of mixed prebiotics made from wheat pollard

It started by filtering wheat pollard with a 20 mash filter to separate wheat pollard from other materials. After that added water up to 45% water content. After mixing evenly, put wheat pollard into the autoclave and heated to 121°C for 15 minutes. After that, dried the

wheat pollard, in an oven at 50° C for ± 48 hours, then mashed the wheat pollard using a disk mill until it was in the form of flour and ready to be used as prebiotic wheat pollard (Utama et al. 2019). The formula for adding 45% water-based on Utama et al. (2017) was as follows:

$$\frac{((material\ water\ content\ (\%) x\ material\ weight\ (g))+A)}{\text{material weight (g)} + \text{A (added water)}}\ x\ 100$$

Making fermented cabbage probiotics

Fermented cabbage probiotics (mixed culture probiotics) begun with cutting the cabbage as thin as possible, then blended and added 8% salt and 6.7% molasses then fermented for 6 days in facultative anaerobes condition (Utama et al. 2018a; 2018b). After that, the results of fermentations were dismantled and ready to be used as probiotics containing *Lactobacillus brevis*, *Lactobacillus plantarum*, *Rhizopus oryzae* and *Saccharomyces cerevise*.

Making mixed culture synbiotics

It started with making probiotics from fermented cabbage juice (Utama et al. 2018a; 2018b). and made prebiotics from wheat pollard (Utama et al. 2019). Prebiotic wheat pollard had 45% water content so it needed to be added with water up to 70% water. The formula for adding 70% water according to Utama et al. (2017) is as follows:

$$\frac{((material\ water\ content\ (\%) x\ material\ weight\ (g))+A)}{\text{material weight (g)} + \text{A (added water)}}\ x\ 100$$

The addition of fermented cabbage by 40% was calculated based on the amount of water added to meet the water content of 70%. Fermentation was carried out for 4 days under facultative anaerobic conditions and stored at room temperature. The fermented wheat pollard (synbiotic mixed culture) was then harvested and dried in a controlled dryer at 40°C for ± 72 hours, after which the fermented wheat pollard (synbiotic mixed culture) was mashed and ready to be used as a feed mixture.

The process of making feed

The feed used contains 20-21% protein with metabolizable energy of 2900-3100 kcal/kg and arranged according to the formulation (Table 1). All feed ingredients were mixed evenly and weighed according to treatment. The feed was given starting from DOC until the age of 8 weeks. The form of feed was uniform in the form of a mash with a size of 20 mash. The composition and chemical composition of the treatment ration is presented in Table 1.

In vivo Test

The DOCs on arrival were weighed to find out the initial body weight then distributed into the experimental cages and gave an isotonic solution with a ratio of 500 ml isotonic: 2 liters of clean water to restore body energy lost during transportation. Each experimental unit contained 8-9 DOCs placed in a cage with 1 x 1 m^2 size. Feed was given according to the needs of the chicken and the residual of the feed were weighed every day. The chickens were weighed every week. Drinking water was given *ad libitum*. The mixed culture probiotics were added during the study (every 4 days) by 50 ml of fermented cabbage probiotics diluted in 500 ml of clean water (10^7cfu/ml). Mixed culture fermented cabbage probiotics contained *Lactobacillus brevis*, *Lactobacillus plantarum*, *Rhizopus oryzae* and *Saccharomyces cerevise* (Utama *et al.*, 2018a; 2018b). The provision of mixed culture fermented cabbage probiotics, with a population of 10^7 cfu / ml probiotic bacteria. No vaccination programs nor the use of antibiotics, drugs, and other additives were applied during the study. The experiment was carried out for 8 weeks.

Parameters Measured

The parameters observed in this experiment were feed consumption, final body weight, body weight gain, feed conversion ratio (FCR), nitrogen retention, income over feed and chick cost (IOFCC), and intestinal villi profile.

Consumption of rations

The amount of ration consumed was obtained from the calculation of the amount of ration consumed every week.

Final body weight

The final body weight was obtained by subtracting the final 8 week weight by initial body weight.

Average Daily Weight Gains

Measurement of body weight gain (ADG) in g/bird/day was calculated following formula:

$$\frac{final\ weight - initial\ weight}{\text{56 days of observation}}$$

Table 1. Composition of treatment ration

Feed Ingredients	Composition of Treatment Ration				
	WP	WPPro	WPPre	WPS40	WPS60
	.....%.....				
Corn	33.00	33.00	33.00	33.00	13.00
Wheat pollard	40.00	40.00	0.00	0.00	0.00
Wheat pollard Prebiotic	0.00	0.00	40.00	0.00	0.00
Wheat pollard Synbiotic	0.00	0.00	0.00	40.00	60.00
Soybean meal	25.00	25.00	25.00	25.00	25.00
VitMin-Mix	0.20	0.20	0.20	0.20	0.20
NaCl	0.25	0.25	0.25	0.25	0.25
L-Lysin HCL	0.10	0.10	0.10	0.10	0.10
DL-Metionin	0.10	0.10	0.10	0.10	0.10
$CaCO_3$	1.35	1.35	1.35	1.35	1.35
Total	100.00	100.00	100.00	100.00	100.00
Crude Protein (%)	20.91[1]	20.91[1]	20.72[1]	20.62[1]	20.74[1]
Metabolic Energy (kcal/kg)	3097[2]	3195[2]	3036 [2]	3126[2]	3148[2]
Crude Fat (%)	2.25[1]	2.25[1]	2.56[1]	2.34[1]	2.49[1]
Crude Fiber (%)	4.23[1]	4.23[1]	4.41[1]	4.10[1]	4.68[1]
Ca (%)	0.84[2]	0.84[2]	0.94[2]	0.91[2]	0.93[2]
P (%)	0.50[2]	0.50[2]	0.58[2]	0.53[2]	0.58[2]
L-Lysin HCl[3]	0.80	0.80	0.80	0.80	0.80
DL-Metionin[3]	0.40	0.40	0.40	0.40	0.40
Resistant Starch[4] (%)	37.25	37.25	37.84	38.18	30.36
Strach[5] (%)	51.63	51.63	51.17	51.46	37.46
Amylose[5] (%)	14.11	14.11	13.45	13.59	8.31
Amylopectin[5] (%)	37.52	37.52	37.72	37.87	29.15
Total Lactic Acid Bacteria (cfu)	-	$2x10^7$	-	$12x10^7$	$12x10^7$
Total *yeast* (cfu)	-	$12x10^8$	-	$24x10^7$	$24x10^7$

[1]: Analysis Results from the Feed and Nutrition Science Laboratory of the Faculty of Animal Husbandry and Agriculture Diponegoro University
[2]: Analysis Result from the Integrated Research and Testing Laboratory of Gajah Mada University
[3]: Analysis Result based on Calculations using a table composition of feed ingredients
[4]: Analysis Results from PAU Gajah Mada University
[5]: Analysis Result from Laboratory of Food Technology and Agricultural Products Gajah Mada University
WP: Wheat pollard based ration, WPPro: Wheat pollard based ration plus probiotic mixed culture, WPPre: Wheat Pollard as prebiotic mixed, WPS40: Wheat pollard as synbiotic mixed culture ration 40%, WPS60: Wheat pollard as synbiotic mixed culture ration 60%

Feed conversion ratio (FCR)

The measurement of feed conversion ratio (FCR) was calculated based on the ratio between the amount of ration consumed and the weight gain measured during the study.

Nitrogen retention

Measurements of nitrogen retention values were performed on 8-week-old chickens. The measurement of nitrogen retention in kampung chickens was carried out by the total collection method. Each group of chickens fed with treatment ration was kept in a battery cage and filled with two chicks per cage and repeated 4 times for each treatment (2 birds per repetition). Six (6) chickens were placed in one cage to get endogenous excreta. All treated chickens were fasted for the first 24 hours to remove the remainder of the feed in the digestive tract. The chickens were given 100g of feed and the excreta were collected for 48 hours. Chickens for collection of endogenous excreta were also fasted for the first 24 hours and provided with drinking water *adlibitum*. The endogenous chicken excreta collected for 44 hours. The excreta were sprayed once every hour with 1N HCl to capture and reduce nitrogen evaporation. The excreta were then dried, ground, and analyzed for nitrogen content. The formula for calculating nitrogen retention (RN) is as follows (Sibbald, 1980):

$$\frac{(FdxNf) - ((E\ x\ Ne) - (En\ x\ Nen))}{(\text{Fd x Nf})} x\ 100$$

Where, RN: Nitrogen Retention (%); Fd: Feed Consumed (g); Nf: Nitrogen Feed (%); Ne: Nitrogen Excreta (%); E: Total Excreta (g); En: Total Ekskreta Endogenous (g); Nen: Nitrogen Endogenous (%).

Income over feed and chick cost (IOFCC)

Income over feed and chick cost is the difference between the average income (in rupiah) obtained from the price of one chicken and the average expenditure of one chicken which includes the price of feed and the price of day old chicken (DOC) during the study.

Profiles of kampung chickens intestinal

The profile of intestinal kampung chickens measured in this experiment were villi length, width, into crypt and crypt width. The morphological profile of chicken's intestines was obtained through all intestinal samples i.e., the duodenum, jejunum, and ileum. About 1 cm^2 for the histological evaluation the intestinal samples were fixed in BNF solution for 24 hours. After fixation, it was then dehydrated with 70, 80, 90, 95% alcohol solution and absolute alcohol I, II, III. Then clarified with xylol I, II, III solution, then the samples were filtered with paraffin I, II, III, and blocked with paraffin. The blocks were cut with a thickness of 4 - 5 µm using a microtome and stained with Hematoxylin-Eosin staining. Measurement of the length and width of villi and measurement of the width and depth of the intestinal crypt were carried out by observing them under a 400x magnification binocular microscope.

Data analysis

The data of ration consumption, final body weight, body weight gain, FCR, income over feed and chick cost (IOFCC), nitrogen retention and intestinal profile were analyzed using a complete randomized design in a unidirectional pattern and if there was a significant effect it was then continued with Duncan's Multiple Range Test (Steel & Torrie 1989). The mathematical model used is as follows:

$$Yij = \mu + \tau_i + \varepsilon_{ij}$$

Where, Y_{ij} = value observed in the i treatment and j replication; µ= the influence of the average value of the general treatment; τ_i= level influence *wheat pollard* treatment I; ε_{ij}= influence the level errors of wheat pollard treatment i and replication j

RESULTS AND DISCUSSION

Results showed that there were significant effects ($P < 0.05$) of the treatment on body weight gain, final body weight, and nitrogen retention as presented in Table 2.

Feed consumption

Probiotic, prebiotic, and synbiotic mixed culture administration did not affect feed consumption. Based on the composition of the ration (Table 1) the fiber content in the treatment feed were fairly the same, as well as the content of amylose, starch, and amylopectin. Utama et al. (2019) stated that the content of hemicellulose, lignin, starch, amylose, and amylopectin in wheat pollard was still considered normal for poultry feed. Consumption of the rations with similar pollard levels (40%) also resulted in the same nitrogen retention.

Average daily weight gain

The effect of probiotics, prebiotics, and synbiotic mixed culture based on wheat pollard had a significant

Tabel 2. Effect of probiotic, prebiotic and synbiotic mixed culture based on wheat pollard on the performances of kampung chickens up to 8 weeks of age

Treatment	Parameter				
	Feed Consumption (g/bird/day)	ADG(g/bird/day)	Final Weight (g/bird)	FCR	Nitrogen Retention (%)
WP[1)]	83.75±12.62	12.32[c2)]±0.63	727.46[c]±35.59	4.05±0.75	80.73[a]±6.52
WPPro	90.37±12.69	13.35[b]±0.61	785.72[b]±34.13	4.03±0.56	82.98[a]±3.48
WPPre	93.20±12.33	12.92[bc]±0.13	766.56[bc]±7.41	4.28±0.54	81.25[a]±1.74
WPS40	83.95±18.57	14.69[a]±0.41	860.86[a]±23.21	3.43±0.83	83.12[a]±1.09
WPS60	88.44±28.12	10.94[d]±0.38	650.62[d]±21.07	4.79±1.64	75.08[b]±2.05
Average	87.94±16.87	12.84±1.38	757.24±24.28	4.12±0.86	80.63±2.98

WP: Wheat pollard based ration; WPPro: Wheat pollard based ration plus probiotic mixed culture; WPPre: Wheat Pollard as prebiotic mixed; WPS40: Wheat pollard as synbiotic mixed culture ration 40%; WPS60: Wheat pollard as synbiotic mixed culture ration 60%;
[a,b,c,d] Different superscript in the same row means significantly different (P<0.05)

effect on body weight gain (P <0.05). The highest body weight gain was found in the group of chicks fed WPS40 treatment i.e., 14.69 ± 0.41 g / bird/day while the lowest body weight gain was found in the WPS60 treatment i.e., 10.94 ± 0.38 g / bird/day. The increase in body weight gain is greatly influenced by the quality of the ration given. The quality of the ration can be seen from nitrogen retention (Table 2). Abdel-Raheem et al. (2012) and Mookiah et al. (2014) stated that the administration of synbiotic ration was very effective in increasing the growth of broiler chickens. The combination of *Bacillus subtilis* and *mannan oligosacharida* (MOS) in broilers caused an increase in the ratio of length to the depth of the crypt in the duodenum and ileum (Sen et al. 2011; Bai et al. 2013).

The WPS40 feed was the best feed from other treatments in facilitating high daily weight gain. The WPS40 was also more efficient compared to other treatments. Feeding a higher dose of synbiotics did not necessarily improve livestock productivity. This can be shown in the treatment of WPS40 and WPS60.

Feed Conversion Ratio (FCR)

Probiotic, prebiotic, and synbiotic mixed culture administration did not affect FCR. The use of mixed culture synbiotic rations improved (P <0.05) the body weight gain even though the FCR value was the same. Microorganisms captured in mixed culture synbiotics were known to help the digestive process by producing several enzymes such as protease, beta-mannanase, and several enzymes that are useful in helping the digestion of feed (Gourbeyre et al. 2011). Sari et al. (2017) reported that the administration of 0 to 5% mixed culture synbiotic in laying hens had no significant effect on egg chemical content and the feed conversion ratio (FCR).

Nitrogen retention

Probiotic, prebiotic, and synbiotic mixed culture administration in the feed, influenced the nitrogen retention parameters significantly (P<0.05). The highest nitrogen retention value (83.12 ± 1.09%) was in group of chicks fed WPS40 treatment while the lowest (75.08 ± 2.05%) was showed in WPS60 treatment. The highest nitrogen retention value in the WPS40 treatment may be due to a better-balanced nutrients component and under the nutrient requirements of the birds as well as the presence of a mixed culture synbiotic that improved the performance of the digestive organs. Utama et al. (2017) stated that food fibers such as arabinosa, mannosa, raffinosa, and resistant starch could modulate microorganisms in the intestine, producing SCFA which had the potential to stimulate the growth of villi. Resistant starch was very effective in producing SCFA especially butyrate in the large intestine and could reduce (Utama et al. 2019). Abdel-Raheem et al. (2012) and Mookiah et al. (2014) also reported that the application of probiotics and prebiotics in broiler diets significantly increased the use of dietary nitrogen.

Income Over Feed and Chick Cost (IOFCC)

Income over feed and chick cost (IOFCC) is an economic variable that illustrates the magnitude of the benefits derived from each treatment. The effect of the use of mixed culture synbiotic rations on income over feed and chick cost in kampung chicken during 8 weeks trial is presented in Table 3.

Tabel 3. Effect of probiotic, prebiotic and synbiotic mixed culture based on wheat pollard based on income over feed and chick cost (IOFCC) of kampung chicken up to 8 weeks of age

Treatments	Revenue from Chicken Selling (IDR)	Expenditures during rearing (Feed+DOC) (IDR)	IOFCC (IDR)
WP[1)]	25,461	19,691	5,770[b2)]±481
WPPro	27,500	21,366	6,135[b]±306
WPPre	26,655	21,831	4,824[b]±251
WPS40	30,130	21,191	8,939[a]±482
WPS60	22,772	21,976	795[c]±881
Average	26,504	21,211	5,293 ±480

WP: Wheat pollard based ration; WPPro: Wheat pollard based ration plus probiotic mixed culture; WPPre: Wheat Pollard as prebiotic mixed; WPS40: Wheat pollard as synbiotic mixed culture ration 40%; WPS60: Wheat pollard as synbiotic mixed culture ration 60%;
[a,b,c,d] Different superscript in the same row means significantly different (P<0.05)

Feeding probiotic, prebiotic, and synbiotic mixed culture to kampung chickens significantly influenced (P<0.05) the Income over feed and chick cost (IOFCC). The highest IOFC was in group of chicken fed WPS40 treatment, i.e., of 8939±482 (IDR/bird) while the lowest was in WPS60 treatment i.e., 795±881 (IDR/bird). Factors affecting IOFCC included the price of rations, consumption of rations, final body weight, and the selling price of chickens per kg of live weight. The production price of wheat pollard-based on synbiotic mixture culture ration was IDR5000/kg while the selling price of live chickens at the end of the treatment period was IDR 35000/bird. Feed containing WPS40 was more efficiently utilized compared to the commercial diet as the price of the commercial ration was expensive (IDR8500/kg).

Histomorphology of the intestine of Kampung chicken age 8 week

The use of wheat pollard mixed culture-based synbiotic ration affected length, width, crypt width, and duodenal crystalline depth, jejunum, and ileum (P<0.05). The effect of giving probiotic, prebiotic, and synbiotic mixed culture based on wheat pollard on the intestinal profile of kampung chicken at 8 weeks old is presented in Table 4.

Villi are an absorptive place and secretion of digestive enzymes so it is assumed that the size of the villi will affect the level of feed digestibility. The length of villi in the duodenum was the longest in the group of chicken fed WPS40 treatment (1959 ± 47μm) while the shortest in the WPS60 treatment (1491 ±76μm). Whilst the length of villi in the jejunum was the longest in the group of chicken fed WPPre treatment (1521 ± 71μm) and the shortest in the WP treatment (1182 ± 67μm). The length of villi in the ileum was the longest in the WPPre treatment (1984 ± 58μm) while the shortest was in the WP treatment (1595 ± 45μm). In general villi length of the group of chicken fed synbiotic culture mixture treatment was 40% better than control, probiotic, prebiotic, and commercial feed treatments chickens groups. Hidayat et al. (2016) stated that intestinal histomorphology reflected the health status of livestock. The increase in villi length, villi width, crypt depth, and crypt width in the intestine was an indication of the growth and health status of livestock.

This condition reflected the healthy digestive tract that comes from probiotic metabolism. *Lactobacillus* creates acidic conditions and produces antimicrobials (free fatty acids, low pH, and bacteriocin), competition for attachment locations in the intestinal epithelium and stimulation of the immune system to protect livestock from pathogenic bacteria by lowering the pH of the intestinal part hindsight thereby disrupting the growth of these bacteria (Tellez et al. 2006; Hill et al. 2014).

The intestinal profile was presented in Table 4 and Figure 4. The nitrogen retention of WPS40 treatment was 83.12 ± 1.09% with the depth and width of the crypts better than other treatments. The width of intestinal in birds fed the synbiotic treatment was longer than other treatments. This cannot be separated from the two components of a synbiotic composition, namely mixed culture probiotics and prebiotic mixed culture.

Utama & Setiani (2014) stated that synbiotic was a fermentation product that came from an increase in the ability of probiotic bacteria caused by the availability of specific prebiotics to be fermented. Starch resistance plays a role in stimulating the speed of crypts cell production. The depth and width of the crypts treatment of WPS40 were better compared to other treatments. Utama et al. (2017) and Utama et al. (2019) stated that food fibers such as arabinosa, mannosa, raffinosa and resistant starch could modulate microorganisms in the intestine, produced short chain fatty acids (SCFA) which had the potential to stimulate the growth of villi and reduced ammonia.

The width of the villi in the duodenum and jejunum showed no significant difference between the groups of treated chicken, whilst the ileum was significantly

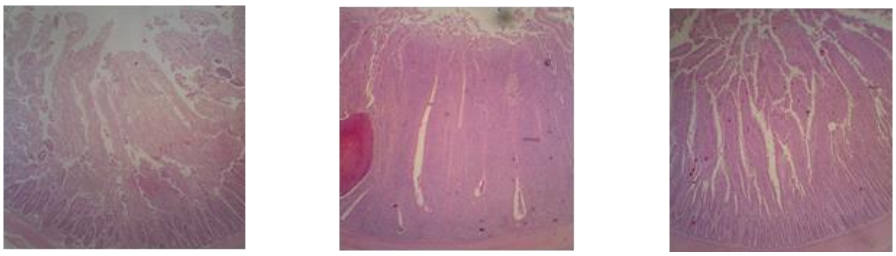
Tabel 4. Influence of probiotic, prebiotic and synbiotic mixed culture based on wheat pollard on the profile of small intestinal of Kampung chickens at 8-weeks-old

Parameters	Treatments				
	WP[1)]	WPPro	WPPre	WPS40	WPS60
	..... μm.....				
Villi length					
Duodenum	1,738^{b}±65	1,781 ^{ab}±53	1,760 ^{ab}±75	1,959 ^{a} ±47	1,491^{c} ±76
Jejunum	1,182^{c}±67	1,308^{bc}±58	1,521^{a}±71	1,454^{ab}±67	1,266^{bc}±62
Ileum	1,595^{b}±45	1,758^{b}±67	1,984^{a}±58	1,802^{ab}±56	1,667^{b}±58
Top width of villi					
Duodenum	257±26	245±24	275±33	252±29	221±15
Jejunum	226±24	216±29	240±25	231±32	195±28
Ileum	125^{c}±34	143^{bc}±36	161^{ab}±27	180^{a}±18	136^{c}±18
Bottom width of villi					
Duodenum	297^{ab}±10	262^{bc}±22	324^{a}±23	313^{a}±31	242^{c}±28
Jejunum	262±27	313±13	266±19	293±25	252±16
Ileum	233^{b}±17	238^{b}±25	330^{a}±31	349^{a}±36	317^{a}±25
Crypts depth					
Duodenum	370±33	417±18	449±37	440±52	383±36
Jejenum	442^{b}±18	330^{c}±20	497^{a}±21	456^{ab}±26	330^{c}±47
Ileum	301±14	311±13	319±41	339±18	323±16
Crypts width					
Duodenum	45^{c}±7	61^{b}±6	66^{b}±5	81^{a}±3	62^{b}±5
Jejenum	43^{b}±4	53^{b}±5	55^{b}±7	80^{a}±2	70^{a}±6
Ileum	44±5	50±7	53±8	54±6	50±6

WP: Wheat pollard based ration; WPPro: Wheat pollard based ration plus probiotic mixed culture; WPPre: Wheat Pollard as prebiotic mixed; WPS40: Wheat pollard as synbiotic mixed culture ration 40%; WPS60: Wheat pollard as synbiotic mixed culture ration 60%
[a,b,c,d] Different superscript in the same row means significantly different (P<0.05)

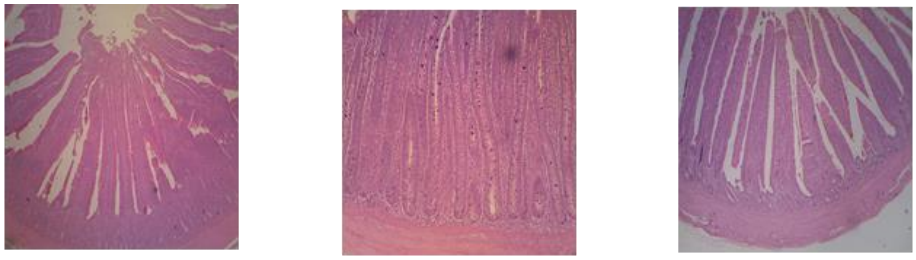
different (P<0.05). The chicks fed WPS40 treatment was significantly different the width of villi from the treatment of WPPro, WP, and WPS60. The bottom width of the villi showed a marked difference in the duodenum and ileum while the jejunum was not significantly different. At the bottom width of villi of the kampung fed WPPre and WPS40 treatments had the same effects. The depth of crypts on jejunum showed a significant difference (P<0.05) between treatment groups while on the duodenum and ileum was no difference. The highest crypt depth showed at WPPre and WPS40 treatments while the lowest was showed at WPPro and WPS60. Probiotics triggered the production of SCFA which played a role in the process of intestinal epithelial cell proliferation (Hung et al. 2012).

The width of the crypts in the duodenum and jejunum of all treatment groups showed a significant difference (P<0.05) whereas in the ileum was not significantly different. The width of the crypts in the duodenum and jejunum of the chicken group fed WPS40 treatment was significantly different from the groups either fed WPPro, or WP, or WPPre, and or WPS60. Pelicano et al. (2005) stated that the villous profile greatly influenced the feed rate so that it affected the absorption of feed nutrients. New epithelial and enterocyte cells were produced by crypts which then migrate to the villi (Perić et al. 2010; Abdel-Raheem et al. 2012; Gómez et al. 2012). The surface of the intestinal tract was coated by viscoelastic mucous gel which acts as a natural defense system and also helps in the absorption of nutrients. In detail, the image of intestinal villi can be seen in figures 1 to 6.



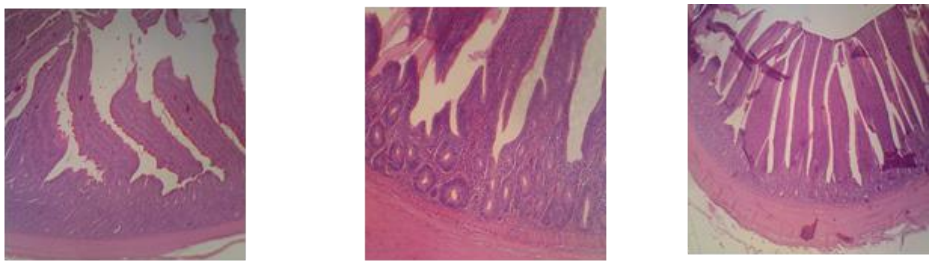
Duodenum Jejunum Ileum

Figure 1. Profile of small intestinal villi of chickens fed wheat pollard (WP) at 8-week-old at 400x magnification



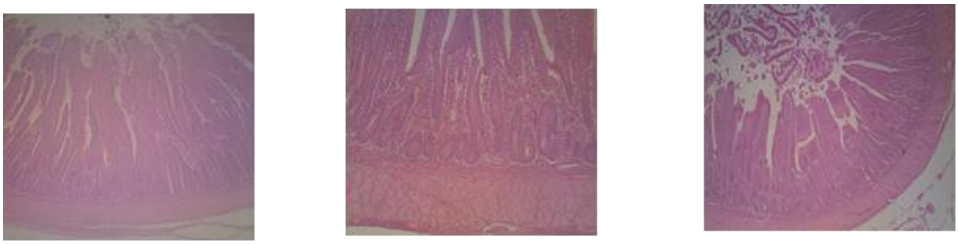
Duodenum Jejunum Ileum

Figure 2. Profile of small intestinal villi of chickens fed Wheat pollard plus probiotic mixed culture-based ration (WPPro) at 8-weeks-old at 400x magnification



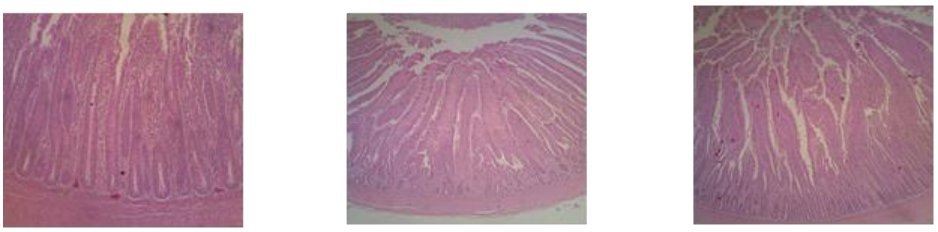
Duodenum Jejunum Ileum

Figure 3. Profile of small intestinal villi of chickens fed Wheat Pollard probiotic mixed culture-based ration (WPPre) at 8-weeks-old at 400x magnification



Duodenum Jejunum Ileum

Figure 4. Profile of small intestine villi of chickens fed wheat pollard synbiotic feed mixed culture 40% (WPS40) at 8 weeks-old at 400x magnification



Duodenum Jejunum Ileum

Figure 5. Profile of small intestine villi of chickens fed wheat pollard feed 60% mixed culture synbiotic (WPS60) at 8 weeks old at 400x magnification

CONCLUSION

It is concluded that the addition of 40% fermented wheat pollard (WPS40) in the ration was able to increase the productivity of kampung chickens until 8 weeks of age as indicated by an increase in body weight, nitrogen retention, intestinal villi profile and IOFCC.

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Jurnal Ilmu Ternak dan Veteriner

Indonesian Journal of Animal and Veterinary Sciences

ISSN: 0853-7380, E-ISSN 2252-696X Date of issue 2020-12-31

UDC: 636.293.2

Abu El-Naser, I.A.M. (Damietta University, Egypt)

Kecenderungan gen langsung dan maternal beberapa sifat produksi dan reproduksi Kerbau Mesir (Direct and maternal genetic trends for some productive and reproductive traits in Egyptian buffaloes)

(Org: Eng)

JITV 25(1): 1-10

This study was done to determine the direct and maternal genetic and phenotypic trends for productive traits such as first lactation milk yield (FLMY, kg), first lactation period (FLP, d) and first lactation daily milk (FLDM, kg), and reproductive traits such as age at first calving (AFC, mo), First days open (FDO, d) and first calving interval (FCI, d). Data were collected over consecutive 25 years (1991 to 2015) of 1104 first lactation of 135 sires and 482 dams maintained at Mahallet Mousa farms of Animal Production Research Institute. Data were analyzed by Animal model to determine genetic parameters for studied traits. Means of FLMY, FLP, FLDM, AFC, FDO and FCI were 1546.5 kg, 189 days, 7.9 kg, 37.9 months, 120.8 days and 428 days, respectively. The direct heritability (h^2a) for same traits were 0.25, 0.18, 0.24, 0.45, 0.18 and 0.19, respectively. Corresponding maternal heritability (h^2m) for mentioned traits was 0.12, 0.19, 0.22, 0.25, 0.12 and 0.12, respectively. Genetic correlations (r_g) among studied traits were varied between -0.19 to 0.38. Accuracy of predicted breeding value varied between 69 to 94, 0.37 to 94 and 42 to 91% for FLMY, FLP, FLDM, AFC, FDO and FCI of sires, cows and dams, respectively that revealed the genetic improvement could be actualized through each of cows or sires or dams. Additive and maternal genetic, permanent environmental and phenotypic trends were not significant for all studied traits. It indicated that it is important to set up a plan to improve genetic and environmental conditions thus, increasing productivity and realization of high profitability.

(Author)

Key Words: Buffaloes, Direct and Maternal Genetic Trends, Phenotypic Trends.

UDC: 631.523

Naimi, M. (Islamic Azad University, Fars, Iran)
Shariati, M. (Islamic Azad University, Kazerun, Iran)
Naeimi, S. (Islamic Azad University, Kazerun, Iran)
Edalatmanesh, MA. (Edalatmanesh MA)

Pengaruh N-acetylcystein terhadap ekspresi gen ERK pada jaringan ovarium tikus dewasa yang mendapat perlakuan akrilamida (Effect of N-acetylcystein on ERK gene expression in ovarian tissue of acrylamide-treated adult rats)

(Org: Eng)

JITV 25(1):11-18

Acrylamide (AA) is a toxic and carcinogenic compound produced in cooking process. The purpose of this study is to evaluate the effect of N-acetylcysteine (NAC) on extracellular signal-regulated kinase (ERK) gene expression level and ovarian histopathological changes in AA-treated rats. Thirty-six female adult Wistar rats were randomly divided into 6 groups including control, positive control (+VE Con), negative control (-VE Con), experimental 1 (Exp1), experimental 2 (Exp2) and experimental 3 (Exp3). Twenty eight days after the treatment, ERK gene expression level was measured by real-time PCR method and ovarian histopathological changes were evaluated. The ERK gene expression level was significantly decreased in the +VE Con, Exp1 and Exp2 groups as compared to the control group ($p<0.05$), but not in the -VE Con and Exp3 groups ($p>0.05$). Histologically, the +VE Con group showed a significant decrease in the number of primary, secondary and Graafian follicles as well as corpus luteum as compared to the control group ($p<0.05$), but not in the negative, Exp2 and Exp3 groups ($p>0.05$). In the Exp1 group, the number of primary and secondary follicles as well as corpus luteum significantly decreased ($p<0.05$), however, the numbers of Graafian follicle and the corpus luteum were significantly increased as compared to the +VE Con group ($p<0.05$). The AA was supposed to increase the apoptosis and folliculogenesis degradation in the rat ovarian tissue by decreasing ERK gene expression. Administration of NAC ameliorated the deleterious effects of AA in a dose-dependent manner and improve folliculogenesis by reducing apoptosis level. Thus, the NAC supplement could be helpful in ameliorating animal fertility.

(Author)

Key Words: Acrylamide, Apoptosis, ERK, Female Rat, N-acetylcysteine

UDC: 592.53

Nesti, DR. (Gadjah Mada University, Yogyakarta)
Baidlowi, A. (Gadjah Mada University, Yogyakarta))
Fauzi, A. (Gadjah Mada University, Yogyakarta)
Tjahajati, I. (Gadjah Mada University, Yogyakarta)

Pengaruh kombinasi campuran bakteri dan jamur pada kualitas fisik fermentasi pakan suplemen berbasis kulit kacang terhadap kualitas fisik dan parameter fermentasi rumen secara *in vitro* (Effect of Mix culture bacteria and fungi in fermented peanut hulls-based feed supplement on physical quality and *in vitro* rumen fermentation parameters)

(Org: Eng)

JITV 25(1): 19-25

The purpose of this research was to determine the effect of the combination of mix culture bacteria (EM4®:E) and fungi (*Trichoderma viridae*:TV) on physical quality and *in vitro* rumen fermentation parameters of peanut hull-based feed supplements. Basal feed was divided into four treatments, which were: P0 (BF); P1 (E:25%+TV:75%); P2 (E:50%+TV:50%),; and P3 (E:75%+TV:25%); and each treatment had three replications. Feeds were fermented facultative anaerobically for nine days. The observed parameters were physical qualities (color, odor, fungi appearance and pH), and *in vitro* rumen fermentation parameters (rumen pH, microbial protein content, and total volatile fatty acid content). Data were analyzed using one way ANOVA design, and the significance of differences were tested using Kruskall-Wallis test for the physical qualities data and Duncan's New Multiple Range Test (DMRT) test for *in vitro* rumen fermentation parameters. Results showed that the combination of E and TV at different level did not affect odor and the fungi appearance parameters (P≥0.05), however, it significantly affected P≤0.05) color change from blackish (1.64) in P0 to brownish in P1, P2 and P3 of 2.44; 2.69; and 2.80, respectively. The pH also decreased significantly (P≤0.05) by 10.67%. Treatment also did not affect the rumen pH, microbial protein content, and total volatile fatty acid content (P≥0.05). It is concluded that the combination of the EM4® 25%: *Trichoderma viridae* 75% on peanut-hull based feed supplement fermentation gives the best result on color and pH fermented feed product without affecting the rumen fermentation process.

(Author)

Key Words: EM_4®, *In vitro* Rumen Fermentation, Peanut Hull, Physical Quality, *Trichoderma viridae*

UDC: 599.735.52

Ginting, SP. (IGRS, Nort Sumatera)
Tarigan, A. (IGRS, Nort Sumatera)
Simanihuruk, K. (IGRS, Nort Sumatera)
Antonius. (IGRS, Nort Sumatera)
Solehuddin. (IGRS, Nort Sumatera)

Pengaruh dua sumber energi berbeda dalam pakan komplit terhadap performans dan metabolit darah kambing Boerka laktasi (Effects of two different energy sources in total mixed diets on the performances and blood metabolites of lactating Boerka goats)

(Org: Eng)

JITV 25(1): 26-33

Lactating goats are prone to negative energy status due to increased body fat reserve mobilization to support the high energy requirement of milk production. The study was aimed to investigate the responses of the lactating goat on diets provided in total-mixed ration differing in the energy sources. The experiment was conducted using a total of 35 does having 2-3 parities and an average bodyweight of 30.3±4.48 kg and BCS of 2.5 ± 0.05 on a scale basis of 1 to 5. Animals were allocated to one of five dietary treatments (seven animals/treatment) formulated to be iso-nitrogen dan iso-calory in a total mixed ration. Cassava meal was used as the source of glucogenic energy and bergafat as the main source of lipogenic energy. There were no DM intake differences (P>0.05) between the glucogenic diet (1.49% and 2.28% fat), but significant increases (P<0.05) in DM intake were observed in goats fed lipogenic diets (fat content range from 4.7 to 7.5%). All animals gained during the lactation period with ADG ranged from 15 to 46 g, indicating that all experimental animals were in positive energy balances. Goat receiving more glucogenic diets gained least and having higher FCR compared to those receiving lipogenic diet (P<0.05). Body condition scores were also improved in lactating goat received more lipogenic diets. Blood glucose and blood urea concentration was not affected by diet treatments and lactation period (P>0.05) and ranged from 37 to 43 mg/dl and 39 to 51 mg/dl, respectively. Numerically, however, the blood glucose and urea level linearly increased as the diet becoming more lipogenic due to the increased feed intake. It is concluded that lactating goats offered diets with lipogenic energy sources (7.5% fat content) presented in pelleted total mixed-ration during the entire lactation period had a higher dry matter and nutrient intakes, body weight gain and body condition score compared to those fed diets with glucogenic energy source.

(Author)

Key Words: Glucogenic Diet, Goat Performance, Lactation, Lipogenic Diet

UDC: 636.597

Kurniawan D. (University of Kahuripan Kediri, Kediri)
Christie, CDY. (University of Kahuripan Kediri, Kediri)

Pengaruh pemberian tepung *Morinda citrifolia* dan *Arthrospira plattensis* terhadap kinerja dan kualitas karkas itik pedaging (The effect of *Morinda citrifolia* and *Arthrospira plattensis* Powder on Performance and Quality of Broiler Duck Carcasses)

(Org: Eng)

JITV 25(1): 34-38

This study was aimed to investigate performance and quality of broiler duck carcasses fed with *Morinda citrifolia* and *Arthrospira plattensis*. A total of 168 two-week-old broiler duck with an initial average body weight of 463 ± 29.38 g and a diversity of 6.35 % were randomly allotted to 7 experimental groups with 4 replications each with 6 bird per replication. Treatments were T0 (basal diet as a control), T1 (basal diet + 0.2% of *Morinda citrifolia* powder (MP)), T2 (basal diet + 0.5% of *Arthrospira plattensis* powder (AP)) , T3 (basal diet + 0.2% of MP+ 0.5% of AP), T4 (basal diet + 0.4% of MP + 0.5% of AP), T5 (basal diet + 0.2% of MP + 0.1% of AP), T6 (basal diet 0.4% of MP + 0.1% of AP). Variables measured were feed intake, body weight gain, feed conversion ratio, carcass percentage, abdominal fat, and visceral organ. Data were analyzed for variance based on a Completely Randomize Design and continued with Duncan's multiple Range Test for differences. Result showed that the treatments did not affect (P>0.05) on feed intake, body weight gain and feed conversion ratio. The treatments also did not affect (P>0.05) carcass percentage, abdominal fat, and

visceral organ. The diet did not significantly improve performance and quality of broiler duck carcasses.

(Author)

Key Words: Broiler Ducks, Performance, Carcass Quality

UDC: 5**75.17**

Prihandini PW. (IBCRS, Pasuruan)
Primasari A. (IBCRS, Pasuruan)
Luthfi M. (IBCRS, Pasuruan)
Efendy J. (IBCRS, Pasuruan)
Pamungkas D. (IBCRS, Pasuruan)

Keragaman genetik sitokrom *b* mitokondria DNA pada populasi sapi asli dan lokal (Genetic diversity of mitochondrial DNA cytochrome *b* in Indonesian native and local cattle populations).

(Org: Eng)

JITV 25(2): 39-47

Information on the genetic diversity of native and local cattle in Indonesia is vital for the development of breeding and conservation strategies. This study was aimed to assess the genetic diversity and phylogenetic relationship of the Indonesian native (Bali) and local [(Donggala, Madura, Sragen, Galekan, Rambon, dan Peranakan Ongole Grade x Bali (POBA)] cattle populations. Genomic DNA was extracted from blood samples (n= 75). Partial sequences of mtDNA cyt *b*, 464 bp, were amplified using the polymerase chain reaction technique (forward primer: L14735 and reverse primer: H15149). Thirty-four reference sequences of *Bos taurus*, *Bos indicus*, and *Bos javanicus* were included in the phylogenetic analyses. A total of 55 polymorphic sites and 13 haplotypes were observed in the whole breeds. No variable sites of mtDNA cyt *b* were observed in Galekan (kept in BCRS) and Rambon cattle. Overall haplotype diversity and nucleotide diversity were 0.515 ± 0.070 and 0.0184 ± 0.0045, respectively. The highest (0.092) and the lowest (0.000) genetic distances were between Bali and Donggala cattle populations and among Galekan (kept in BCRS), Rambon, and POBA cattle populations, respectively. Both mtDNA network and phylogenetic analyses revealed two major maternal lineages (A and B) of the studied population. Most of the sampled individuals (69.33%, present in haplotype H8-H19) were linked to lineage B, which belonged to the same cluster with *Bos javanicus*. Overall, most of the Indonesian native and local cattle populations had a considerable genetic diversity and shared a common maternal origin with *Bos javanicus*.

(Author)

Key Words: Indonesian Cattle, Cytochrome *b*, Genetic Diversity, Phylogenetic Analysis

UDC: 636.39

Anggraeni A. (IRIAP, Bogor)
Saputra F. (IRIAP, Bogor)
Hafid A. (IRIAP, Bogor)
Ishak ABL. (IRIAP, Bogor)

Pengaruh non-genetik dan genetik sifat pertumbuhan pada saat lahir hingga umur 120 hari dari kambing G_2 Sapera (Non-genetic and genetic effects on the growth traits from birth to 120 days of age of G_2 Sapera goat)

(Org: Eng)

JITV 25(2): 48-59

Information on non-genetic and genetic factors is required in the selection program. Indonesian Research Institute for Animal Production (IRIAP) has been conducting a selection of the growth traits of Sapera goat (50% Saanen, 50% PE). This research was aimed to study non-genetic and genetic effects on growth traits from birth to the age of 120 days old of the 2nd generation (G_2) of Sapera goat. Data on body weight and measurement were collected from kids at birth (105 head.) to the age of 120 days old (51 head). The 30 days interval growth data were calculated by linear interpolation. Non-genetic effects were analyzed by General Linear Model for unbalanced data by considering sex, type of birth, the month of kidding, and year of kidding as fixed variables. The genetic component was analyzed by a mixed linear model by considering sire as a random variable. Heritability was estimated by the paternal half-sib method. Non-genetic factors mostly had no significant effect ($P > 0.05$) on body weight and measurement. The 90 days old and 120 days old males had higher weights than females ($P<0.05$). Birth type and year of kidding had significant effects ($P<0.05$) on body weight and some measurements at certain ages. No significant months of kidding effect on the growth traits ($P>0.05$). Heritability values of body weight (h^2 = 0.11-0.19) and body sizes (h^2 = 0.03-0.24) were relatively low. Except high heritability values for birth weight and for body weight at 30 days old (h^2 = 0.59 and 0.29), and for hip girth at 30 days old and at 60 days old (h^2 = 0.13-0.54). The growth traits of G_2 Sapera kids were affected by sex and year of kidding and slightly influenced by genetic (sires) factors.

(Author)

Key Words: Dairy Goat, Growth, Genetic, Non-Genetic

UDC: 636.32/.38

Mardenli O. (University of Aleppo, Syria)
Mohammad MS. (University of Alqadisiah, Iraq)
Hassooni HA. (University of Al-Muthanna, Iraq)

Efisiensi dimetil sulfoksida dan etilena glikol pada perkembangan subsequen embrio domba Awassi yang divitrifikasi (Efficiency of dimethyl sulphoxide and ethylene glycol on subsequent development of vitrified Awassi sheep embryos)

(Org: Eng)

JITV 25(2): 60-67

The use of cryoprotectants in vitrification would reduce the critical damages to the embryos, thus increase the survival rates. This research was conducted in the laboratory of reproductive biotechnology at the faculty of Agriculture of Aleppo University. The study aimed to evaluate the viability and survivability of early Syrian Awassi embryos under the influence of dimethyl sulphoxide (DMSO) and ethylene glycol (EG) following vitrification. Embryos were vitrified in

three solutions of cryoprotectants (A: DMSO (3 ml), B: EG (3 ml), and C which was composed of a combination of DMSO (1.5 ml) and EG (1.5 ml)). After thawing, embryos that had been vitrified in C solution achieved the highest rates of cleavage (P< 0.01) comparing with A and B solutions for 2-16 cell stage (50.00% Vs 30.77% and 36.36%), morula (9.00% Vs 44.44% and 40.00%) and blastocyst stage embryos (92.86% Vs 58.33% and 50.00%) respectively. Down to the hatching blastocyst stage, 2-16 cell stage vitrified embryos in C solution achieved an encouraging rate comparing with A and B solutions (39.20% Vs23.08% and 22.73% respectively). The rates of arrested embryos decreased significantly (P< 0.05) after thawing across the three solutions especially the morula and blastocyst stage (0.00 and 3.70% respectively) (C solution). No significant differences were observed in the three types of embryos across all stages and solutions despite the large range among these rates. Given the apparent benefit of the participatory effect of cytoprotectants, it is advised to use a mixture of DMSO and EG (1:1) in vitrification of ovine embryos.

(Author)

Key Words: Awassi Sheeep, Dimethyl Sulphoxide, Ethylene Glycol, *In Vitro* Embryo Production, Vitrification

UDC: 591.16

Zakiya NAH. (University of Tanjungpura, Pontianak)
Yanti AH. (University of Tanjungpura, Pontianak)
Setyawati TR. (University of Tanjungpura, Pontianak)

Daya simpan semen cair kambing Peranakan Etawah dalam tris yang disubstitusi dengan sumber energi berbeda (Viability of Peranakan Etawah liquid semen preserved in tris substituted with various energy sources)

(Org: Eng)

JITV 25(2): 68-73

The use of liquid semen for artificial insemination program of Etawah crossbreed goat (PE) is an alternative to replace frozen semen which is constrained by limited and expensive facilities. Production of liquid semen is faster than frozen semen, but the viability of liquid semen which preserved with a standard extender such as tris egg yolk is very short. The purpose of this study was to determine the viability of PE goat semen in egg yolk tris substituted with energy sources such as glucose, galactose, and mannose and to determine the most efficient energy source for semen preservation. This research was conducted from August to September 2018 at the Artificial Insemination Center in Lembang, West Java. This study was designed in a randomized block design (RBD) consist of three experimental groups divided into five groups. Fresh semen of PE goats were preserved using extender which energy source has been modified. Results showed that using glucose in PE goat semen extender produced the best motility among other groups (64.29 ± 9.2%). The highest viability was found in extender with fructose substitution (86.76 ± 2.3%). The longest viability of liquid semen was found in the extender with glucose substitution. It lasted for six days.

(Author)

Key Words: Glucose, Liquid Semen, Motility, PE Goat, Viability

UDC: 591.53

Zuratih. (ICARD, Bogor)
Budhi SPS. (Univercity of Gadjah Mada, Yogyakarta)
Bachruddin Z. (Univercity of Gadjah Mada, Yogyakarta)

Kondisi fermentasi rumen dengan penambahan bekatul fermentasi dalam pakan secara *in vitro* (Condition of rumen fermentation as ımpacted by supplementation of fermented rice brand using ın vitro gas production technique)

(Org: Eng)

JITV 25(2): 74-80

Methane is one of the gases produced by ruminants during feed fermentation in the rumen. This experiment was aimed to investigate the production of monacolin K in rice bran fermented by Monascus purpureus mold and the influence of the supplementation of fermented rice bran using Monascus purpureus mold on elephant grass basal diet on fermentation products and methane production in an in vitro gas production method. The study consisted of two experiments. The first experiment analysis of monacolin K production in fermented rice bran using Monascus purpureus. Fermentation is done by the addition of Monascus purpureus at levels 0, 4, 8, and 12% (v/w) of substrate (rice bran) with 3 replications. Monacolin K in the substrate was analyzed using HPLC. The second experiment was the evaluation of supplementation of fermented rice bran to elephant grass basal diet using in vitro gas production. The treatment diet evaluated were Pennisetum purpureum (control), Pennisetum purpureum:rice bran (1:1 ratio), and Pennisetum purpureum:rice bran fermented. Each treatment was replicated 3 times. Results from the first experiment shows that rice bran with the highest monacolin K content was in rice bran fermented at 12% by Monascus purpureus. Result from the second experiment showed that supplementation of fermented rice bran to Pennisetum purpureum basal diet did not affect rumen ammonia concentration, VFA, protein microbial production, and dry matter and organic matter digestibility. However, methane production (CH_4) was reduced (P<0.05) by 50%, and the protozoal population was decreased (P<0.05) by 80%. It is concluded that supplementation of fermented rice brands containing monacolin K was able to reduce methane production and the protozoa population without affecting feed fermentation.

(Author)

Key Words: Fermentation, *In Vitro*, Methane, Monacolin K, *Monascus purpureus*

UDC: 577.181

Sinurat AP. (IRIAP, Bogor)
Pasaribu T. (IRIAP, Bogor)
Purwadaria T. (Atma Jaya Catholic University of Indonesia, Jakarta)
Haryati T. (IRIAP, Bogor)
Wina E. (IRIAP, Bogor)
Wardhani T. (IRIAP, Bogor)

Evaluasi biologis zat bioktif beberapa tanaman sebagai imbuhan pakan pengganti antibiotik pada ayam broiler (Biological evaluation of some plant bioactives as feed additives to replace antibiotic growth promoters in broiler feeds)

(Org: Eng)

JITV 25(2): 81-90

Antibiotics (AGP) have been used as feed additives to promote growth and feed efficiency in poultry production. However, many countries include Indonesia now ban the use of AGP and attempts are made to replace the antibiotic to maintain good performances of broilers. Plant bioactives is one of the alternatives that could replace the AGP. An experiment was conducted in an attempt to replace the AGP in broiler feed with a mixture of some plant bioactives (liquid smoke of cashew nutshell, *Phyllanthus niruri*, and clove leaves). Eight (8) dietary treatments were formulated to have similar nutrients consist of negative control (NC), positive control (NC+AGP), diets supplemented with liquid plant bioactives in 3 levels and diets supplemented with powder plant bioactives in 3 levels. Each diet was fed to 6 replications of 10 birds each from 1 to 35 days old. The performances and the immune response of the broilers due to the treatments were observed. Results showed that the powder plant bioactives could not improve the performance of broilers. None of the feed additives (AGP or plant bioactives) affect the immune systems of the broilers. However, liquid plant bioactives in low dose improved the performance of broilers better than the AGP and therefore is suitable to replace the antibiotic as feed additives in broiler diet.

(Author)

Key Words: Antibiotic Growth Promoters, Broilers, Plant Bioactives

UDC: 615.35

Masoumi F. (Islamic Azad University, Kazerun, Iran)
Shariati M. (Islamic Azad University, Kazerun, Iran)
Mokhtari M. (Islamic Azad University, Kazerun, Iran)

Pengaruh sari ekstrak *Silybum marianum* dan *l-carnitine* pada perubahan stereologi hati tikus yang diberikan perlakuan diazinon (Effects of *Silybum marianum* aqueous extract and l-carnitine on stereological changes in diazinon-treated rat liver.)

(Org: Eng)

JITV 25(2): 91-98

As an organophosphorus, Diazinon (DZN) impairs liver tissue function by inhibiting acetylcholinesterase and causing oxidative stress. In this study, the effects of *Silybum marianum* aqueous extract (SMAE) and L-carnitine (LC) on the stereological and histopathological changes of the liver in DZN-treated male rats were investigated. The rats in this study were placed into 9 groups of 8 each containing control, placebo, and a combination of DZN, SMAE, and LC. The animals received SMAE and chemicals orally for 30 days. At last, the liver tissue of all animals was removed. Then, tissue sections from the liver were provided to study the stereological markers including liver volume and weight, hepatocytes' volume, central venous volume, sinusoidal volume, connective tissue volume, inflammation rate, and a number of the hepatocytes' nuclei. Also, the sample tissues were evaluated histopathologically. Treatment with DZN significantly reduced the liver volume and weight, hepatocyte volume, central venous volume, sinusoidal volume, and hepatocyte nucleus number compared to placebo and control but it significantly increased the inflammation and volume of liver's connective tissue. However, co-administration of SMAE and LC with DZN improved liver volume and weight, hepatocyte volume, central venous volume, sinusoidal volume, connective tissue volume, and hepatocyte nucleus number alone compared to the DZN treatment. Liver inflammation was also significantly decreased compared to the DZN treatment but comparing to the placebo and control groups, it increased significantly. Simultaneous administration of SMAE and LC has protective effects on liver tissue and can reduce DZN-induced liver injury in rats.

(Author)

Key Words: Diazinon, L-carnitine, Liver, Rat, *Silybum marianum*

UDC: 613.287.5

Asmarasari SA. (IPB University, Bogor)
Sumantri C. (IPB University, Bogor)
Gunawan A. (IPB University, Bogor)
Taufik E. (IPB University, Bogor)
Anggraeni A. (IRIAP, Bogor)

Variasi genetik gen protein susu dan hubungannya dengan komponen susu sapi Friesian Holstein (Genetic variants of milk protein genes and their association with milk components in Holstein Friesian cattle)

(org: Eng)

JITV 25(3): 99-111

Protein content in milk is an important indicator of milk. Accordingly, genetic improvement to produce Holstein Friesian (HF) dairy cattle is important. The objective of this study was to evaluate the genetic variant of milk protein genes and its effect on milk component traits of Holstein Friesian (HF). A total of 100 HF were used in this study. The HF cattle used have physiological status in the lactation period 1 up to 3 and lactation change of 1 up to 12 months. Genotype variants of milk protein genes were identified using Real Time-Polymerase Chain Reaction method. Analysis of milk component was carried out covering the component of protein, fat, lactose, and solid non-fat (SNF) by using a milk quality measuring device (Lactoscan). Genotyping of cattle blood samples consisted of DNA extraction, genes amplification using the RT-PCR method. The result showed that protein milk was significantly affected ($p<0.05$) by the genetic variants of CSN1S1-192 and CSN2-67 genes. Fat milk was significantly affected ($p<0.05$) by the genetic variants of CSN1S1-192 and CSN3 genes. Meanwhile, solid non-fat milk was significantly affected ($p<0.05$) by the genetic variants of CSN-BMC9215, CSN-BMC6334, CSN1S1-14618, CSN2_67, and CSN3 genes. Lactose milk was significantly affected ($p<0.05$) by the genetic variants of

CSN-BMC9215 and CSN2-67 genes. It was concluded that genetic variants of the milk protein genes have an association with the component of cow's milk (protein, fat, solid non-fat, and lactose).

(Author)

Key Words: Genetic Variant, Protein Genes, Milk Component

UDC: 591.463.1

Rasad SD. (Padjadjaran University, Sumedang)
Solihati N. (Padjadjaran University, Sumedang)
Winangun K. (Padjadjaran University, Sumedang)
Yusrina A. (Padjadjaran University, Sumedang)
Avicenna F. (Padjadjaran University, Sumedang)

Pengaruh waktu inkubasi selama proses *sexing* sperma pada sperma kualitas Pasundan Bull (Effect of incubation time during sperm sexing process on sperm quality of pasundan bull)

(Org: Eng)

JITV 25(3): 112-119

The research was conducted to evaluate the effect of incubation time on viability, plasma membrane integrity, abnormality, and DNA integrity of sexed Pasundan's bulls sperm. The sperm sexing used 5% and 10% concentrations of Bovine Serum Albumin (BSA). A completely randomized design with three treatments and six replications was used in this study. The data were analyzed using variance analysis followed by Duncan's multiple distance test. Parameter evaluated were sperm longevity, plasma membrane integrity (PMI), abnormality, and DNA integrity of sexed Pasundan bulls sperm. Results showed that incubation time gave significant effect ($P<0.05$) on the longevity of sperm, but not on the PMI of Pasundan bulls sexed sperm. The incubation time of 45 minutes gave the highest value of longevity sperm on the upper layer (4.33 days) and the lower layer (4.17 days). Furthermore, the abnormality of sperm X in the upper layer was 4.00%-4.20% and the lower layer was 4.10%-4.40%. Meanwhile, the DNA integrity of an upper layer was 98.16%-98.66%, and the lower layer was 97.83%-98.58%. It is concluded that 45 minutes of incubation time significantly affected the longevity of sperm, but not plasma membrane integrity, abnormality, and DNA integrity of Pasundan bulls sexed sperm.

(Author)

Key Words: Incubation Time, Pasundan Bull, Sexed Sperm

UDC: 636.39

Pamungkas FA. (IPB University, Bogor)
Purwanto BP. (IPB University, Bogor)
Manalu W. (IPB University, Bogor)
Yani A. (IPB University, Bogor)
Sianturi RG. (IRIAP, Bogor)

Aplikasi termografi inframerah sebagai indikator dalam penentu kondisi fisiologis dan hematologis kambing perah sapera dara (Use of infrared thermography for identifying physiological and hematological conditions of young sapera dairy goats)

(Org: Eng)

JITV 25(3): 120-130

Infrared thermography (IRT) is an alternative solution that can be applied to replace invasive methods currently used in the monitoring of goats' physiological and hematological parameters. This study was done to compare and correlate the physiological and hematological conditions of young Sapera dairy goats and their correlations with results obtained by IRT. Four young Sapera dairy goats (weight of 26-28 kg) were kept in the individual rearing cage. Skin surface temperature (TS), rectal temperature (TR), body temperature (TB), heartbeat (HR), respiration rate (RR), and IRT at eyes, mouth, nose, legs, left body, right body, vagina, and vulva were monitored from 6 a.m. to 6 p.m. in 2 h intervals. Blood samplings were done at the beginning and the end of the obsevation time. Results showed that IRTs at several body parts were positively correlated with physiological parameters, except for heartbeat. Negatively correlation was observed in hematological parameters. The highest correlation ($r = +0.85$) was observed in the correlation between the results of the left rear leg IRT on TB. It was concluded that IRT can be applied to examine goats' physiological conditions especially body temperature.

(Author)

Key Words: Thermography, Infrared, Physiological Parameter, Hematological Parameter, Dairy Goat

UDC: 591.3

Mardenli O. (University of Aleppo, Syria)
Mohammad MS. (University of Alqadisiah, Iraq)
Alolo AY. (University of Aleppo, Syria)

Pengaruh kombinasi ukuran folikel, FSH dan sisteamin pada embrio domba produksi *in vitro* (Effect of combination of follicle size, FSH and cysteamine on *in vitro* production sheep embryos)

(Org: Eng)

JITV 25(3): 131-138

The participatory relationship among the follicle size, follicle stimulating hormone (FSH), and cysteamine (antioxidant agent) contribute to the production of embryos characterized by abundance and good quality. The aim of this study was to evaluate the efficacy of FSH, cysteamine and follicle size on *in vitro* embryo production of Awassi sheep oocytes. Follicles sizes were determined into two groups: small follicles (1-2 mm) and large follicles (> 2 mm). Oocytes were matured across two increasingly shared levels of FSH and cysteamine: A (40 ng/ml + 50 µM) and B (60 ng/ml + 100 µM). Results of the bilateral interaction showed significant differences across the follicle size (large follicles group) and the maturation treatment (B medium) in the rates of fertilization (highest value: 67.51%; p= 0.02), cleavage (highest value: 65.41%; p= 0.01), 2-16 cell stage (lowest value: 2.29%; p= 0.0001), blastocyst stage (highest value:

44.82%; p= 0.04), down to morula stage arrest (lowest value: 55.17%; p= 0.04) and Type I embryos (highest value: 52.87%; p= 0.03). Likewise, matured oocytes of small follicles group (B medium) attained the highest rate of morula stage (56.60%; p= 0.03). No significant differences were observed in Type II and Type III embryos. In order to obtain high yields of good quality embryos, it is advised to add FSH and cysteamine with levels of 60 ng/ml and 100 µM respectively to maturation medium of ovine oocytes obtained from follicles with a diameter > 2mm.

(Author)

Key Words: Cysteamine, Follicle Size, FSH, *In Vitro* Embryo Production, Sheep

UDC: 613.2.038

Saenab A. (ICARD, Bogor)
Wiryawan KG. (IPB University, Bogor)
Retnani Y. (IPB University, Bogor)
Wina E. (IRIAP, Bogor)

Pengaruh sinergistik biofat dan biochar cangkang biji mete untuk mitigasi metana dalam rumen (Synergistic effect of biofat and biochar of cashew nutshell to mitigate methane in the rumen)

(Org: Eng)

JITV 25(3): 139-146

One way to reduce methane emissions is by using feed additives derived from plant extracts containing secondary metabolic compounds. This study aimed to evaluate the effectiveness of combinations of biofat and biochar (bioindustrial products of cashew nut shells) as feed additive in reducing methane production and improving *in vitro* rumen fermentation. In this experiment, a randomized block design with 6 treatments and 4 replications was applied. The treatments were different combination of biofat (BF) and biochar (BC) as follows: Control= substrate only without addition of biofat or biochar; BFBC1 = 0%BF: 100%BC; BFBC2 = 25%BF:75%BC; BFBC3 = 50%BF:50%BC; BFBC4 = 75%BF:25%BC; BFBC5 = 100%BF: 0%BC. The measured variables were: total gas and CH_4 productions, dry matter (DM); organic matter (OM); and neutral detergent fiber (NDF) ruminal degradabilities, NH_3 and partial volatile fatty acid (VFA) concentrations. Result showed that the addition of combinations of biofat and biochar into the substrates resulted in significant decrease ($P<0.01$) of CH_4 production in the ruminal fluid. Compared to control, CH_4 production was lower by 11.50% (BFBC1), 36.85% (BFBC2), 38.50% (BFBC3), 41.84% (BFBC4) and 26.07% (BFBC5). All combinations except BFBC5 produced similar NH_3 concentration but significantly higher propionate and total VFA concentration in the *in vitro* rumen than control, dry matter degradability and organic matter degradability in the presence of combination of biofat and biochar at different ratios were similar to the control ($P>0.05$). In conclusion, the best combination in producing a synergistic effect as a feed additive to reduce methane, and enhance rumen fermentation products *in vitro* is BFBC4: biofat 75% and biochar 25%.

(Author)

Key Words: Cashew Nut Shell, Biofat, Biochar, Combination, *In Vitro*, Rumen

UDC: 591.16

Sitanggang G. (Directorate General of Livestock and Animal Health, Jakarta)
Arifiantini RI. (IPB University, Bogor**)**
Jakaria J. (IPB University, Bogor**)**

Pengaruh genetik dan non genetik karakteristik semen sapi Bali (*Bos javanicus*) [Genetic and non-genetic effects on semen characteristics of Bali cattle (*Bos javanicus*)]

(Org: Eng)

JITV 25(4): 147-152

The objective of this study was to evaluate effect of genetic and non-genetic factors on semen characteristics including ejaculate volume, sperm concentration, total sperm number and sperm motility of Bali cattle. Semen data were collected from the National Centre of Artificial Insemination at Singosari, Malang, East Java, Indonesia. A total of 3,847 ejaculates of 17 Bali bulls from 2014 to 2016 were collected and evaluated. Data were analyzed by restricted maximum likelihood (REML) method using mixed models which the bull was a random effect, while age of bull, season of collection, frequency of ejaculation and collection intervals were the fixed effects. Results showed that age significantly affected all semen characteristics ($P<0.01$). Season affected only on sperm motility ($P<0.01$). Effect of frequency of ejaculation and collection intervals was significant on all studied variables ($P<0.01$), except sperm motility. Repeatability of ejaculate volume, sperm concentration, total sperm number and sperm motility was 0.43; 0.35; 0.32 and 0.31, respectively. It is concluded that age, frequency of ejaculation and collection intervals were the most factors affected semen characteristics of Bali cattle. Repeatability estimations of semen characteristics were moderate to high.

(Author)

Key Words: Bali Cattle, Non-Genetic, Repeatability, Semen Characteristics

UDC: 577.161.4

Ghorbani Vahed M. (Islamic Azad University, Fars, Iran)
Khanbabaee R. (Islamic Azad University, Qaemshahr, Iran)
Shariati M. (Islamic Azad University, Kazerun, Iran)
Edalatmanesh MA. (Islamic Azad University, Shiraz, Iran)

Peningkatan pengaruh asam lemak omega-3 dalam pematangan oosit secara *in vitro* (Improving the effects of omega-3 fatty acid on the *in vitro* oocyte maturation)

(Org: Eng)

JITV 25(4): 153-161

This research was conducted in order to determine the effects of omega-3 on oocyte in vitro maturation and the level of expression of tribbles (TRIB1, TRIB2 and TRIB3 genes) in cumulus cells. Eight-ten weeks old NMRI mice were super-ovulated using 7.5 IU pregnant mare's serum gonadotropin (PMSG, Intraperitoneal) and they were killed after 44 hours and their ovaries were removed. The oocytes were used for *in vitro* maturation and the cumulus-oocyte complexes (COCs) were released. Cumulus cells and oocytes were assigned into control, ethanol-treated and groups

exposed to 10 and 100 µg/ml of omega-3. The cells were prepared to assess the maturation stage in order to evaluate the gene expression level. The data were statistically analyzed. Exposing oocytes to low dose (10 µg/ml) and high dose (100 µg/ml) of omega-3 resulted in a reduced rate of GV-stage oocytes, decreased MI-oocytes and increased MII-oocytes. The enhanced maturity of COCs was also detected in response to a high dose of omega-3 (100 µg/ml). Exposure of cumulus cells to omega-3 (10 and 100 µg/ml) induced TRIB2 and inhibited TRIB3 gene expression level; however, TRIB1 gene expression level increased and decreased in response to low (10 µg/ml) and high (100 µg/ml) concentrations of omega-3, respectively. The addition of omega-3 to the environment of oocytes or cumulus cells affected the maturation of oocytes and cumulus cells, which was followed by the differential expression of TRIB genes, suggesting that there was a role of fatty acid metabolism in the differentiation and maturation of cumulus cells.

(Author)

Key Words: Cumulus Cells, Maturation, Omega-3, Oocyte, *TRIB*

UDC: 591.16

Yendraliza. (Universitas Islam Negeri Sultan Syarif Kasim Riau, Riau)
Rodiallah M. (Universitas Islam Negeri Sultan Syarif Kasim Riau, Riau)
Astuti T. (University Mahaputra Muhammad Yamin, West Sumatera)
Elfawati. (Universitas Islam Negeri Sultan Syarif Kasim Riau, Riau)

Status Reproduksi dan dynamic populasi sapi Kuantan di Kabupaten Kuantan Singingi (Reproduction status and population dynamic of Kuantan cattle in the Kuantan Singingi Regency)

(Org: Eng)

JITV 25(4): 162-172

The purpose of this study was to determine reproductive efficiency, population dynamics, natural increase and estimated output of the Kuantan cattle in the Kuantan Singingi Regency, Province of Riau, Indonesia. A total of 311 Kuantan cattle and 99 Kuantan cattle farmers were used in this study through a survey study. Respondent samples were taken from seven districts. Data sampling using purposive sampling with survey methods. Data collection was carried out by interviewing farmers and observing and was analyzed descriptively. Parameters measured were reproductive efficiency, natural increase, estimated output and population dynamics of Kuantan cattle. Results showed that the reproductive efficiency of Kuantan cattle was 1.04%, natural increase 5.14%, the balance of male and female 1: 5, the value of male NRR 50% and female NRR 100.56%, total cattle out 18.69% and total incoming cattle 18.69%, output value 48.88% and estimated population dynamics 2.85%. In conclusion, Kuantan cattle reproduction has not been efficient with the natural increase of the Kuantan cattle was very low, and the replacement stock availability for male and female cattle has not been fulfilled. It is recommended not to release Kuantan cattle in the next 5 years to maintain population balance.

(Author)

Key Words: Natural Increase, Output, Population Dynamic, Reproduction

UDC: 577.214

Rohmah L. (IPB University, Bogor)
Darwati S. (IPB University, Bogor)
Ulupi N. (IPB University, Bogor)
Khaerunnisa I. (University of Insan Cendekia Mandiri, Bandung)
Sumantri. (IPB University, Bogor)

Mutasi baru gen prolaktin ekson 5 pada ayam IPB-D1 (Novel mutation of exon 5 prolactin gene in IPB-D1 chicken)

(Org: Eng)

JITV 25(4): 173-181

The prolactin gene (PRL) is a gene that controls the incubation and egg production in laying chickens. The nature of incubation will reduce egg production and disrupt the reproductive system in local chickens. The purpose of this study was to identify the polymorphism of prolactin genes in IPB-D1 chickens using the direct sequencing method. The polymorphism of the exon 5 prolactin gene was carried out on 46 samples of IPB-D1 chicken DNA which was a collection of the Division of Animal Genetics and Breeding, Faculty of Animal Science IPB. DNA sequences as a reference for designing exon 5 primers were obtained from the National Center for Biotechnology Information (NCBI) with the GenBank access code: AF288765.2. DNA extraction was carried out using the phenol-chloroform technique. DNA amplification resulted in a PCR product with a size of 557 bp. In this study, the genotype frequency, allele frequency, heterozygosity value and Hardy-Weinberg equilibrium were calculated. The results of the study found 5 SNPs in exon 5, namely g.7823A>G, g.7835A>G, g.7886T>A, g.8052T>C, and g.8069T>C. All SNPs are polymorphic and in Hardy-Weinberg equilibrium except g.8052T>C. The g.7823A>G, g.7835A>G, g.8052T>C SNPs are synonymous mutations that do not change amino acids, while the g.7886T>A and g.8069T>C SNPs are non-synonymous that change amino acids. Both g.7886T>A and g.8069T>C SNPs are potential as a marker assisted selection for the characteristics of egg production in IPB-D1 chickens.

(Author)

Key Words: IPB-D1 Chicken, Mutation, Prolactin Gene, SNP

UDC: 636.58.033

Mareta I. (Diponegoro University, Semarang)
Nathaniel G. (Diponegoro University, Semarang)
Yudiarti T. (Diponegoro University, Semarang)
Widiastuti E. (Diponegoro University, Semarang)
Wahyuni HI. (Diponegoro University, Semarang)
Sugiharto S. (Diponegoro University, Semarang)

Pengaruh kombinasi filtrat buah belimbing wuluh dan terasi terhadap performa, mikroba usus dan profil darah ayam

broiler (Effect of *Averrhoa bilimbi* fruit filtrate and shrimp paste mixture on performance, gut microbes and blood profile of broilers)

(Org: Eng)

JITV 25(4): 182-189

This study was aimed to evaluate effect of a mixture of *Averrhoa bilimbi* fruit filtrate and shrimp paste (*Mysis* sp.) on the growth performance, blood profile, selected intestinal bacterial number and pH value of broiler digestive tract. The mixture of *A. bilimbi* fruit filtrate and shrimp paste were incubated for 4 days and were then used in the experiment. For *in vivo* experiment, 40 day-old Lohmann broiler chicks were distributed randomly to two treatment groups, i.e., control (T1) and chickens given the mixture of 10% *A. bilimbi* fruit filtrate and shrimp paste in drinking water (T2). Body weight and feed intake were recorded weekly. At day 42, 2 birds from each pen (8 chicks per treatment group) were taken for blood and digesta collection. Internal organ weight and carcass traits were determined thereafter. Feed conversion ratio (FCR) was lower (P<0.05) on the treatment group than the control. Hemoglobin and mean corpuscular volume (MCV) of the treatment group were higher (P<0.05) than that of the control. Total cholesterol was higher (P<0.05) in the treatment group than that in control. Total cecum coliform was lower (P<0.05) in the treatment group than that in the control. The pH values of the small intestinal segments (jejunum, ileum, cecum) were lower (P<0.05) in the treatment group than that in the control group. In conclusion, administration of the blends of *A. bilimbi* fruit filtrate and shrimp paste into drinking water improved FCR, increased hemoglobin and MCV values, decreased gut pH and cecal coliform of broiler chickens.

(Author)

Key Words: Broiler Chicken, Mixture *Averrhoa bilimbi* L Filtrate and Shrimp Paste, Performance

UDC: 637.54'659.7

Prayitno AH. (Politeknik Negeri Jember, Jember)
Rahman TH. (Politeknik Negeri Jember, Jember)

Kualitas kimia bakso daging itik afkir (*Anasplathyryncos*) yang disubstitusi filler tepung edamame (*Glycine max* (L) Merril) (Chemical quality of culled duck meatball (*Anas plathyryncos*) substituted with edamame flour (*Glycine max* (L) Merril) filler)

(Org: Eng)

JITV 25(4): 190-194

This study aimed to determine the effect of edamame flour filler substitution on the chemical quality of culled duck meatballs. The research material consisted of culled duck meat, tapioca flour, edamame flour, albumen, garlic, onion, salt, pepper, monosodium glutamate, sodium tripolyphosphate, and ice. The edamame flour filler substitution treatments were P0 (0%), P1 (5%), P2 (10%), P3 (15%), and P4 (20%) from total filler. Each treatment consisted of five replications. The parameters which tested were moisture, protein, fat, fiber, and ash contents. Data on chemical quality results were analyzed by analysis of variance using completely randomized design and if there was significantly different (P<0.01), then it'll be further tested by the Duncan's New Multiple Range Test. Results showed that the substitution of edamame flour filler starting from the level of 5% was significantly affected water content, protein, fat, fiber, and ash of culled duck meatballs. Edamame flour can be used as a filler substitution up to 20% level by giving a good effect on increasing the meatball protein content, but also having a negative impact with increasing the fat content of culled duck meatballs.

(Author)

Key Words: Meatball, Culled Duck Meat, Filler, Chemical Quality, Edamame Flour

UDC: 613.2.038

Utama CS. (Diponegoro University, Semarang)
Zuprizal. (Gajah Mada University, Yogyakarta)
Hanim C. (Gajah Mada University, Yogyakarta)
Wihandoyo. (Gajah Mada University, Yogyakarta)

Pengaruh pemberian probiotik, prebiotik dan sinbiotik *mixed culture* berbasis *wheat pollard* terhadap produktivitas ayam kampung (Effects of probiotics, prebiotics, and synbiotics mixed culture based on wheat- pollard on kampung's chicken productive performance)

(Org: Eng)

JITV 25(4): 195-205

This research was aimed to assess the effectiveness of processed wheat pollard -based rations to increase the productivity of Kampung chickens raised until 8 weeks old. The research was carried out in a completely randomized design with 5 treatments and 4 replications. The treatments consisted of wheat pollard based ration (WP), wheat pollard based ration plus probiotic mixed culture (WPPro), wheat Pollard as prebiotic mixed (WPPre) , wheat pollard as synbiotic mixed culture ration 40% (WPS40), wheat pollard as synbiotic mixed culture ration 60% (WPS60) . The parameters observed were feed consumption, final body weight, feed conversion ratio (FCR), weight gain, nitrogen retention, income over feed and chick cost (IOFCC) and profiles of intestinal villus. Results showed a significant effect of the treatments on the final body weight, weight gain, nitrogen retention, IOFCC and profiles of small intestinal villus (duodenum, jejenum, ileum). It was concluded that the inclusion of 40% wheat pollard synbiotic mixed culture (WPS 40) in the ration was able to increase the productivity of kampung chickens reared until 8 weeks old.

(Author)

Key Words: Wheat Pollard, Kampung Chicken, Synbiotic Mixed Culture, Productive Performance

AUTHOR GUIDELINES

Indonesian Journal of Animal and Veterinary Sciences or IJAVS contains:

(i) Primary scientific manuscript of unpublished research results.

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AUTHOR GUIDANCE

The manuscript is written in proper English, accompanied by an abstract in English and Indonesian. The manuscript is typewritten on the A4 paper size using Times New Roman 10 with single spaces distance and 4 cm from the left side, 3 cm from the right side, 3 cm from the top and bottom sides. We provide you with IJAVS Template that you can find on our website: http://medpub.litbang.pertanian.go.id/index.php/jitv.

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Citation in the references:

Literature in the reference is written alphabetically based on the author's name. The same author is written sequentially starting from earlier order.

Example of reference writing

Primary paper:

Bhanja SK, Anjali DC, Panda AK, Sunder GS. 2009. Effect of post-hatch feed deprivation on yolk-sac utilization and young broiler chickens. Asian-Aust J Anim Sci. 22:1174-1179.

Book:

a. Lawrence TLJ, Fowler VR. 2002. Growth of farm animals. 2nd ed. New York (USA): CABI Publishing.
b. Bamualim A, Tiesnamurti B. 2009. Konsepsi sistem integrasi antara tanaman padi, sawit, dan kakao dengan ternak sapi di Indonesia. In: Fagi AM, Subandriyo, Rusastra IW, penyunting. Sistem integrasi ternak tanaman padi, sawit, kakao. Jakarta (Indones): LIPI Press. p. 1-14.
c. Paloheimo M, Piironen J, Vehmaanpera J. 2010. Xylanases and cellulases as feed additives. In: Bedford MR, Partridge GG, editors. Enzymes in farm animal nutrition. 2nd ed. New York (USA): CABI Publishing. p. 12-53.

Proceeding:

Umiyasih U, Antari R. 2011. Penggunaan bungkil inti sawit dan kopra dalam pakan penguat sapi betina berbasis limbah singkong untuk pencapaian bobot badan estrus pertama >225 kg pada umur 15 bulan. Prasetyo LH, Damayanti R, Iskandar S, Herawati T, Priyanto D, Puastuti W, Anggraeni A, Tarigan S, Wardhana AH, Dharmayanti NLPI, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology.

Bogor (Indones): Indonesian Center for Animal Research and Development. p. 192-199.

Thesis:

Krisnan R. 2008. Kombinasi penggunaan probiotik mikroba rumen dengan suplemen katalitik pada pakan domba (Thesis). [Bogor (Indones)]: Institut Pertanian Bogor.

Electronic magazines:

Wina E, Tangendjaja B, Dumaria. 2008. Effect of *Calliandra calothyrsus* on *in vitro* digestibility of soybean meal and tofu wastes. Livest Res Rural Develop. Vol. 20 Issue 6. http://www.lrrd.org/lrrd20/6/wina 20098.htm.

Institution:

a. [NRC] National Research Council. 1985. Nutrient requirements of sheep. 6th revised. Washington DC (USA): National Academic Press.
b. [CDC] Centers for Disease Control. 2006. Standard operating procedure for the direct Rapid Immunohistochemistry Test (dRIT) for the detection of rabies virus antigen. [accessed December 20th, 2011]. http://www.rabiesblue print.com/IMG/pdf/DRIT_SOP.pdf.

Patent:

Blanco EE, Meade JC, Richards WD. 1990. Ophthalmic Ventures, assignee. Surgical stapling system. United States patent US 4,969,591. 1990 Nov 13.

10. **Citation in text:**

The citation consists of the author's last name and publication year.

Example:

a. One author: ..... grow slower than lamb fed cattle's milk (Supriyati 2012). Supriyati (2012) formulates.....
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Indonesian Journal of Animal and Veterinary Sciences

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Acknowledgement

Editorial board and executive editor of Indonesian Journal of Animal and Veterinary Sciences (IJAVS) extent high appreciation to the expertises of peer reviewer of IJAVS (Volume 25 No. 4 2020).

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Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 25, Number 4, December 2020 ISSN 0853-7380 E-ISSN 2252-696X

LIST OF CONTENT

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