ISSN 0853-7380
E-ISSN 2252-696X

Accredited by the Ministry of Research, Technology, and Higher Education
Decree Number: 21/E/KPT/2018

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 24
Number 1
March 2019

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

JITV	Volume 24	Number 1	Page 1 - 48	Bogor, March 2019	ISSN 0853-7380

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

JITV	Volume 24	Number 1	Page 1-48	Bogor, March 2019	ISSN 0853-7380 E-ISSN 2252-696X

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 Padjajaran Kav. E. 59, Bogor 16128 - Indonesia
Telephone (0251) 8322185
Fax (0251) 8380588
E-mail: criansci@indo.net.id; jitvnak@yahoo.com
Website: http://medpub.litbang.pertanian.go.id/index.php/jitv

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

PREFACE

In this edition, volume 24 No 1, we proudly present articles from animal and veterinary sciences including genetics; reproduction and food technology. The articles published in this edition are:

"The influence of dietary protein and energy levels on the performance, meat bone ratio and meat chemical composition of SenSi-1 Agrinak chicken"; "Phenotypic characteristics of Exotic-broiler, Kampung, Male Exotic-layer, KUB-1 and Pelung chickens"; "The effect of addition selected amino acids in extender semen on quality and DNA stability of frozen-thawed Sumba Ongole bull spermatozoa"; "The relationship of pod colour with the quality of Indigofera zollingeriana"; "Generation of scFv-Monoclonal Antibody Avian Influenza Diagnostic tests"; "Evaluation of surra treatment strategies attacking horses and buffaloes in East Sumba District, Nusa Tenggara Timur Province of Indonesia (2010 – 2016)".

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, March 2019

Complete paper may be accessed through:
http://medpub.litbang.pertanian.go.id/index.php/jitv or
http://peternakan.litbang.pertanian.go.id/index.php?option=com_content&view=article&id=3633&Itemid=119 or
through database CAB DIRECT (www.cabdirect.org) or
Indonesian Scientific Journal Database (isjd.pdii.lipi.go.id)

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 24, Number 1, March 2019 ISSN 0853-7380 E-ISSN 2252-696X

LIST OF CONTENT

Influence of Dietaryprotein and Energy Levels on Performance, Meat: Bone Ratio, and Meat Chemical Composition of SenSi-1 Agrinak Chickens

Hidayat C, Iskandar S

Indonesian Research Institute for Animal Production, Ciawi Bogor Indonesia
E-mail : hidayat_c2p@yahoo.com

(received 06-12-2018; revised 17-02-2019; accepted 18-02-2019)

ABSTRAK

Hidayat C, Iskandar S. 2019. Pengaruh kandungan protein dan energi metabolis ransum terhadap kinerja produksi, rasio daging terhadap tulang dan komposisi kimia daging ayam SenSi-1 Agrinak. JITV 24(1): 1-8. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1913

Sensi-1 Agrinak merupakan hasil pemuliaan dari salah satu jenis ayam lokal di Indonesia untuk produksi daging. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh tingkat energi metabolis dan protein dalam ransum terhadap kinerja produksi, rasio daging terhadap tulang, dan komposisi kimia daging ayam SenSi-1 Agrinak yang dipelihara sampai umur 10 minggu. Dua ratus enam belas *unsexed* DOC Sensi-1 Agrinak diberi enam ransum perlakuan dengan kandungan protein kasar (PK) (21,19 dan 17%), serta kandungan energi metabolis (EM) (2800 dan 3000 kkal/kg). Setiap kombinasi perlakuan diulang sebanyak empat kali. Dalam setiap kombinasi perlakuan ada sembilan unsexed-DOC. Parameter yang diamati adalah kinerja produksi (Bobot hidup, konsumsi ransum, viabilitas, FCR), indeks ekonomi (EPEF), rasio daging terhadap tulang, dan komposisi kimia daging. Hasil penelitian menunjukkan bahwa peningkatan kandungan PK ransum meningkatkan bobot hidup dan EPEF (P<0,05). Ayam Sensi-1 Agrinak memiliki FCR terbaik (2,59), ketika diberi pakan yang mengandung 21% PK dan 3000 kkal/kg. Peningkatan kandungan PK ransum dapat meningkatkan rasio daging terhadap tulang pada potongan karkas bagian dada, paha atas, dan paha bawah. Sementara itu, peningkatan kandungan PK dan EM ransum tidak mempengaruhi (P>0,05) komposisi kimia daging. Dapat disimpulkan bahwa kadar protein kasar dan energi metabolis yang optimal untuk ayam Sensi-1 Agrinak untuk masa pemeliharaan 0-10 minggu adalah 21% PK dan 3000 kkal/kg.

Kata Kunci: Energi Metabolis, Protein, Ayam Sensi-1 Agrinak

ABSTRACT

Hidayat C, Iskandar S. 2019. The influence of dietary protein and energy levels on the performance, meat bone ratio and meat chemical composition of SenSi-1 Agrinak Chicken. JITV 24(1): 1-8. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1913

Sensi-1 Agrinak is a strain of the improved native chickens for meat production in Indonesia. The objective of this study was to investigate influence of different dietary energy and protein levels on performance, meat bone ratio, and meat chemical composition of Sensi-1 Agrinak chicken, reared until 10 weeks of age. Two hundred and sixteen of unsexed day old chickens (DOC) of Sensi-1 Agrinak were subjected to six experimental rations differed in dietary crude protein (CP) content,. Namely: 21;19; and 17 % and dietary metabolizable energy (ME) (2800 and 3000 kcal/kg). Each treatment combination was replicated four times and fed from day old to 10 weeks old. In each treatment combination there were nine unsexed-DOCs. The parameters observed were performance (i.e. live weight, feed intake, viability, FCR), economic index (European Production Efficiency Factor/EPEF), meat bone ratio, and meat chemical composition. Result showed that increased of dietary CP level increased live weight and EPEF (P<0.05). Sensi-1 Agrinak chicken had the best FCR (2.59), when fed diet containing 21% CP and 3000 kcal/kg. Increased dietary CP level increased the meat-bone ratio of breast, thighs, and drumsticks. Meanwhile, increased levels of dietary CP and ME did not affect (P>0.05) meat chemical composition. It is concluded that optimal dietary levels of crude protein and energy for unsexed Sensi-1 Agrinak chicken up to 10 weeks of age were 21% CP and 3000 kcal/kg.

Key Words: Dietary Metabolizable Energy, Dietary Protein, Sensi-1 Agrinak Chicken

INTRODUCTION

Improving and developing Indonesian native chicken has been continued done by the Indonesian government in order to increase the contribution of native chicken to the national poultry meat. Supported by the general view of the Indonesian people who generally consider that native chicken meat is more delicious than the broiler chicken meat, as indicated by the price of native chicken meat is more expensive. There is a significant potential in developing native chicken in Indonesian livestock industry Chen et al. (2008) stated that generally, native chicken meat has intense flavour, firm texture, low fat content and rich in other nutrients. One of the improved strains of native chicken in Indonesia as a meat producer is Sensi-1

Agrinak chicken. The Sensi-1 Agrinak chicken was selected for optimum growth of market demand, by the average live weight of 1 kg per bird at 10 weeks of age (Hasnelly et al. 2017). Nonetheless, information of the influence of dietary protein nor energy on the performance of Sensi-1 Agrinak chicken has not yet reported.

Niu et al. (2009) stated that energy and protein were the main nutrients with the highest economic value compared to other nutrients. Energy is needed by animals to support all of their activities. Meanwhile, protein is a key component of cells that plays an important role in the life process. Accordingly, optimal chicken production requires an appropriate combination of dietary metabolizable energy (ME) and crude protein (CP). This is to ensure maximum utilization of each and every nutrient of the diet and help to reduce the cost of production (Zaman et al. 2008). One method of determining protein requirements and ration energy in local chickens is through feeding trial testing using several treatment diets varying protein and energy content. As was done by Magala et al (2012) in uganda local chickens, using several experimental diets varying 18-20% crude protein and 2800-3000 kcal metabolizable energy content per kg. Meanwhile, Hidayat et al. (2017) used diet varying 17-19% CP and 2800-3100 kcal ME/kg to determin the most efficient diet to an improved breed of Indonesian native chicken. The nutritional content of the feed greatly influenced the quality of chicken meat (Marcu et al. 2011a). Marcu et al. (2011b) explained that the quality of chicken carcass was determined by the maximum proportion of meat and the minimum proportion of bone in the carcass cut. Meanwhile, the main chemical components of meat (protein, fat) are important indicators in determining meat quality (Diaz et al. 2010). The objective of the current study was to investigate the effect of feed with different energy and protein levels on the performance, meat bone ratio, and meat chemical composition of Sensi-1 Agrinak chickens reared to 10 weeks of age.

MATERIALS AND METHODS

Two hundred and sixteen of unsexed DOCs of Sensi-1 Agrinak chicken were subjected to six experimental diets, differed in combination of dietary crude protein (CP) (21, 19, and 17%) and dietary ME 2800 and 3000 kcal/kg. Each treatment combination was replicated four times, arranged in a (3 x 2) completely factorial design. In each treatment combination there were nine unsexed DOCs, randomly picked and confined in a colony wire cage with floor space of 35 x 35 cm and height of 40 cm. Heating light bulb was constructed to provide proper heat during brooding age. Feed in mash form and drinking water were provided *ad libitum*.

Health programs such vaccination with proper vaccines and cage sanitation were applied following the programs that usually applied to exotic broiler chicken breed. Immunization program consisted of: i) Against NDIB (new castle disease and infectious bronchitis) at four days of age ; ii) Against IBD (gumboro) at seven days old; iii) The IBD vaccine was repeated in day 21; iv) ND-IB was repeated at the age of 28 days. Feed composition and nutrients content of the experimental diets are presented in Table 1.

Live weight and feed intake were measured every week. Mortality was recorded at any time when the loss happened. At the age of ten weeks, one male and one female from each replication were taken randomly for carcass and carcass cuts analysis. Slaughter process was applying the Islamic slaughter method (Hafiz et al. 2015). Feed conversion ratio was calculated by feed consumed divided by the total live weight gain of bird (g feed consumption/ g live weight gain). European Production Efficiency Factor (EPEF) was calculated using this formula (Marcu et al. 2013):

$$EPEF = \frac{\text{Viability (\%) x Live Weight (kg)}}{\text{Age (day) x FCR}} \times 100$$

Viability : Percent of the number of chickens that live in each replication
Live weight : Live weight at the time of measurement (g/bird)
Age : Age at the time of observation is stopped (day)
FCR : Feed Conversion Ratio

Fresh meat bones ratio was calculated in grams meat per one gram bone (Marcu et al. 2013), using the formula below :

Fresh meat: bones ratio (g meat : 1 g bone) = meat weight / bones weight

After deboning, laboratory analysis for chemical composition of meat (moistures, proteins, lipids, and cholesterols) was carried out at the accredited laboratory in the Indonesian Research Institute for Animal Production (IRIAP) of the Indonesian Agency for Agricultural Reseach and Development (IAARD) Ministry of Agriculture. The raw data were processed using methods of biostatistics with Microsoft Excel spreadsheet application. Analysis of variance (ANOVA) of SAS 9.13 statistical program was used to test the statistical significance of differences among treatment mean values.

Table 1. Feed composition and nutrients content of experimental diets fed to SenSi-1 Agrinak chicken up to 10 weeks of age

Feedstuffs	21%CP		19%CP		17%CP	
	3000 kcal ME/kg	2800 kcal ME/kg	3000 kcal ME/kg	2800 kcal ME/kg	3000 kcal ME/kg	2800 kcal ME/kg
Corn, (%)	38.65	37.65	31	38	30.6	44
Rice bran, (%)	23.5	27.5	39.43	33.15	45	32.65
Soybean meal, (%)	30	23.5	20.72	14	13.75	6
Meat bone meal, (%)	3	8.5	5	12	6.8	15
Vegetable oil, (%)	3	0.5	2	0.5	2	0.5
CaCO3, (%)	0.7	1.2	0.7	1.2	0.7	0.7
NaCl (%)	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix, (%)	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix, (%)	0.15	0.15	0.15	0.15	0.15	0.15
Lysine, (%)	0.15	0.15	0.15	0.15	0.15	0.15
Choline clorida, (%)	0.1	0.1	0.1	0.1	0.1	0.1
Sodium bicarbonate, (%)	0.1	0.1	0.1	0.1	0.1	0.1
Total, (%)	100	100	100	100	100	100
Nutrient content						
Protein, (%)*	21	21	19	19	17	17
Metabolizable Energy/ME, (kcal/kg)**	3054	2891	3050	2890	3057	2875
Ratio ME:Protein	145	137	160	152	179	169
Moisture, (%)***	10.51	10.79	10.54	9.62	9.75	9.89
Crude Fibre, (%)***	4.04	4.42	4.79	4.38	4.28	3.73
Abu, (%)***	8.77	8.74	8.16	10.61	9.17	9.86
Calcium, (%)***	1.33	1.22	0.86	1.66	0.97	1.62
Phosphor, (%)***	1.06	0.97	0.93	1.24	1.17	1.19
Amino acid content***						
Asparagine, (%)	2.56	2	2.03	1.7	1.67	1.36
Serine, (%)	1.04	0.85	0.87	0.86	0.74	0.68
Glutamine, (%)	4.29	3.38	3.41	2.94	2.83	2.57
Glycine, (%)	1.2	1.17	1.16	1.84	1.08	1.57
Histidine, (%)	0.62	0.49	0.5	0.45	0.45	0.4
Arginine, (%)	1.67	1.4	1.4	1.37	1.23	1.26
Threonine, (%)	0.91	0.73	0.75	0.68	0.65	0.58
Alanine, (%)	1.24	1.07	1.14	1.16	1	1.12
Proline, (%)	1.41	1.23	1.26	1.32	1.1	1.31
Cystein, (%)	0.17	0.13	0.16	0.12	0.13	0.12
Tyrosine, (%)	0.67	0.56	0.55	0.55	0.46	0.44
Valine, (%)	1.24	0.99	1.07	0.93	0.9	0.84
Methionine, (%)	0.29	0.22	0.25	0.25	0.21	0.21
Lysine, (%)	1.57	1.25	1.34	1.09	1.07	0.85
Isoleucine, (%)	1.01	0.79	0.81	0.67	0.67	0.57
Leucine, (%)	1.97	1.57	1.66	1.43	1.41	1.33
Phenylalanine, (%)	1.12	0.91	0.89	0.8	0.8	0.72

* Calculated based on crude protein content obtained from proximate analysis in the laboratory of feedstuffs used

** Calculated based on estimates of the metabolizable energy content of the used feedstuffs with the calculation of gross energy x 0.725 (NRC 1994)

*** Analysis results in the laboratory

RESULTS AND DISCUSSION

Performance responses of live weight, feed consumption, viability, and European Production Efficiency Factor (EPEF) were presented in Table 2. ANOVA results on these variables did not show any significant interaction effect (P>0.05). Therefore the comparissons were applied separately for each treatment factors.

Sensi-1 Agrinak chicken live weight, feed intake, viability and European Production Efficiency Factor (EPEF) were not influenced by metabolizable energy (ME) level of feed during whole experimental period (P<0.05). Meanwhile, increasing dietary CP levels increased live weight and EPEF (P<0.05), but did not affect feed intake and the viability. These findings were in line with those presented by Liu et al. (2015) who reported that there was an increase in live weight of Chinese indigenous chicken (Lueyang Black-boned Chickens), due to an increase in dietary CP levels. This results may have indicated that the dietary ME of 2800 kcal/kg has fulfilled the dietary energy requirements of Sensi-1 Agrinak chicken reared up to 10 weeks of age.

Feed whith feed contained 21% CP significantly (P<0.05) resulted in the highest live weight (897 g/bird) and the EPEF (462). Therefore, Sensi-1 Agrinak chicken looks more precise and more economical to be fed with high CP content (21%), as seen from the highest achievement on live weight and EPEF compared to the other dietary protein treatments. Results in the present study was also similar to Nguyen et al. (2010) who reported that increased dietary CP content (15 to 21%) resulted in an improvement of growth performance of Thailand indegenous chicken (Betong chicken). Dewi et al. (2015) explained that increased dietary CP level would increase the protein retention, hence, increasing growth. This was due to the increasing number of proteins that would be used to prepare the chicken body tissues. The range of ME content (at 2800-3000 Kal/kg) and CP contents (at 17%, 19.21%) in the feed did not affect the viability level of Sensi-1 Agrinak chickens. The present study showed that the feed with low dietary protein content (17%) was considerably safe for Sensi-1 Agrinak chicken until 10 weeks age resulted in viability of more than 95%.

Feed intake of Sensi-1 Agrinak chickens up to 10 weeks of age was not influenced by the dietary ME and CP level. This is in agreement with findings of Raphulu & Rensburg (2018) who also reported no differences in feed intake of South Africa indigenous chicken (Venda village chickens) with varying ME (11;11.7;12 MJ/kg) and CP levels (14;17;19%). Contrastly, Banerjee et al. (2013) reported that increased levels of protein of feed increased the level of feed intake of other South Africa indigenous chicken (Koekoek chickens). Raphulu & Rensburg (2018) stated that the effect of dietary protein on feed intake in poultry species was inconsistent due to great variation in body weight, age, genotype, sex, and stage of maturity among native chickens.

Effect of dietary treatments on FCR, efficiency of dietary CP consumption (Table 3) showed that, there was significant interactions (P<0.05) between dietary CP and ME. The FCR of Sensi-1 Agrinak chicken was

Table 2. Live weight, feed consumption, viability, and European Production Efficiency Factor (EPEF) of Sensi-1 Agrinak chicken given experimental diets up to 10 weeks of age

Factors	Live weight (g/bird)	Feed intake (g/bird	Viability (%)	EPEF
Metabolizable Energy (ME)				
2800 kcal/kg	838[a]	2335[a]	95.41[a]	396[a]
3000 kcal/kg	847[a]	2280[a]	98.16[a]	428[a]
Pooled SE	29.83	45.06	2.92	28.5
Crude Protein (CP)				
17%	805[a]	2261[a]	97.25[a]	385[a]
19%	825[a]	2311[a]	95.87[a]	390[a]
21%	897[b]	2350[a]	97.25[a]	462[b]
Pooled SE	38.69	64.97	4.50	38.95
Interaction				
ME x CP	NS	NS	NS	NS

Values in the same column and factor, with different superscript are significantly different (P<0.05)
Pooled SE = Pooled Standard Error
NS = Not statistically significant (P>0.05)

the best FCR (2.59), when given feed with high ME (3000 kcal/kg) and high CP (21%). However, when the ME of the diet was low (2800 kcal/kg), there was no significant (P>0.05) effect of protein levels on FCR. When the efficiency of dietary protein consumption was calculated, the diet with 17% protein gave the best (64%) efficiency, although it was not in agreement with the efficiency of energy consumption, which was the lowest (9.08 kcal/g LWG). The treatment which resulted in the best FCR (treatment with 21% CP and 3000 kcal/kg), was suspected to be caused by the best utilization efficiency of dietary ME (7.76 kcal/g LWG). On the contrary, combination of 17%CP and 3000 kcal ME/kg gave the low FCR (2.99 g feed/g LWG). This study seemed somehow in harmony with the research reported by Magala et al. (2012), showing that cockerels fed the low-protein and metabolizable energy diet (18% CP and 2800 kcal/kg) would respon in superior protein efficiency ratio in comparison to that of chicken fed on the high-protein and metabolizable energy diet (20% CP and 3000 kcal/kg).

Eventually, these results indicated that Sensi-1 Agrinak chicken showed the ability to convert feed to meat better when fed with high nutrient density feed.

There was no significant (P>0.05) interaction between CP and ME on meat : bone ratio of the breast, thighs, and drumsticks of Sensi-1 agrinak chicken (Table 4).

Breast, thighs, and drumsticks are the most valuable chicken carcass cuts in the Indonesian market, even small differences in price of the three carcass cuts above, it can have significant economic impact. Globally, the breast, thighs, and drumsticks, were widely used as an indicator of the quality of carcasses in meat chickens (Marcu et al. 2013). The higher meat : bone ratio has a meaning of a higher chicken carcass quality, which shows the higher proportion of meat of the whole carcass. Meat : bone ratio of breast, thighs, and drumstick did not affected by increasing dietary ME level. Meanwhile, an increase in dietary CP level increased the meat : bone ratio in the three carcass cuts (breast, thighs, drumsticks) as shown in Table 5. This result showed that higher dietary CP caused high CP consumed, and eventually increased the protein retention to be converted to body flesh .

There was no significant (P>0.05) interaction effect between dietary CP and dietary ME on the meat chemical compositions parameters (moisture, protein, fat, cholesterol) of Sensi-1 Agrinak chicken (Table 5). This finding was different from report of Marcu et al. (2012), who explained that dietary nutrition was an external factor as a major influence on the chemical composition of chicken meat. Previously, Bogosavljevi-Bošković et al. (2010) also reported that the chemical composition of chicken carcass cuts were influenced by genetic and non-genetic factors.

The current study showed that the protein and fat content of Sensi-1 Agrinak chicken meat was consistent with that reported by several researchers that the protein and fat content of meat chicken was above 22.50% CP and below 3% fat (Marcu et al. 2011b; 2009; Horniakova et al. 2009). The cholesterol content of Sensi-1 Agrinak chicken meat was not affected by dietary CP and ME levels. The current study was in tune with the result of study which shown by Maliwan et al. (2017) in Thai indigenous crossbred chicken (Korat chicken) which reported that the feeding program was given different dietary CP levels (15,17,19,21% CP) during the growth period (0-10 weeks), affected no different meat chemical composition (moisture, protein, and cholesterol). Brewer et al. (2012) reported that studies which had been done to attempt with several feeding programmes using many dietary CP levels, showed that all the programs did not affect meat quality. The present study showed that the content of fat at Sensi-1 Agrinak chicken meat was lower than the fat content of broiler chicken meat as reported by Hasanuddin et al. (2013), who showed that the fat content of broiler chickens meat was in the range of 4.88-7.38%. Contrastly, the content of cholesterol at Sensi-1 Agrinak chicken meat was higher than the content of cholesterol of broiler chickens meat,i.e., in the range of 0.046 – 0.049% (Abdulla et al. 2015).

Table 3. Feed Conversion Ratio (FCR), and efficiency of dietary crude protein and dietary metabolizable energy consumptions of Sensi-1 Agrinak chicken given experimental diets up to 10 weeks of age

Variable	3000 kcal ME/kg			2800 kcal ME/kg			SEM
	21% CP	19% CP	17% CP	21% CP	19% CP	17% CP	
FCR (g feed/g LWG)	2.59^{a}	2.83^{b}	2.99^{b}	2.84^{b}	2.99^{b}	2.86^{b}	0.043
Efficiency of crude protein consumption, (g/g LWG)	0.54^{bc}	0.54^{bc}	0.50^{a}	0.64^{d}	0.56^{c}	0.50^{ab}	0.010
Efficiency of ME consumption, (kcal/g LWG)	7.76^{a}	8.61^{bc}	9.08^{c}	8.25^{ab}	8.89^{c}	8.20^{ab}	0.117

SEM = Standar error of mean
LWG = Live wight gain
Values in the same row with different superscript are significantly different (P<0.05)

Table 4. Meat bone ratio on breast, thighs, and drumsticks of 10 weeks old unsexed Sensi-1 Agrinak chicken given experimental diets

Factors	Breast	Thighs	Drumsticks
Metabolizable Energy (ME)			
2800 kcal/kg	4.40[a]	5.29[a]	3.00[a]
3000 kcal/kg	4.88[a]	4.72[b]	2.85[a]
Pooled SE	0.425	0.239	0.135
Crude Protein (CP)			
17%	3.97[b]	4.72[b]	2.90[ab]
19%	4.46[b]	4.93[ab]	2.79[b]
21%	5.49[a]	5.36[a]	3.09[a]
Pooled SE	0.44	0.30	0.16
Interaction			
ME x CP	NS	NS	NS

Values in the same column and factor, with different superscript are significantly different (P<0.05)
Pooled SE = Pooled Standard Error
NS = Not statistically different (P>0.05)

Table 5. Chemical composition *(as is)* of meat of 10 weeks age-old of Sensi-1 Agrinak chicken fed experimental diets

Factors	Moisture (%)	Protein (%)	Fat (%)	Cholesterol (%)
Metabolizable Energy (ME)				
2800 kcal/kg	74.05[a]	24.24[a]	1.97[a]	0.16[a]
3000 kcal/kg	74.12[a]	24.15[a]	2.31[a]	0.17[a]
Pooled SE	0.31	0.53	0.26	0.01
Crude Protein (CP)				
17%	73.60[b]	24.19[a]	1.83[a]	0.15[a]
19%	74.08[ab]	24.32[a]	2.23[a]	0.18[a]
21%	74.57[a]	24.07[a]	2.36[a]	0.18[a]
Pooled SE	0.38	0.75	0.40	0.01
Interaction				
ME x CP	NS	NS	NS	NS

Values in the same column and factor, with different superscript are significantly different (P<0.05)
Pooled SE = Pooled Standard Error
NS = Not statistically different (P>0.05)

The current study showed that the protein and fat content of Sensi-1 Agrinak chicken meat was consistent with that reported by several researchers that the protein and fat content of meat chicken was above 22.50% CP and below 3% fat (Marcu et al. 2011b; Marcu et al. 2009; Horniakova et al. 2009). The cholesterol content of Sensi-1 Agrinak chicken meat was not affected by dietary CP and ME levels. The current study was in tune with the result of study which shown by Maliwan et al. (2017) in Thai indigenous crossbred chicken (Korat chicken) which reported that the feeding program was given different dietary CP levels (15,17,19,21% CP) during the growth period (0-10 weeks), affected no different meat chemical composition (moisture, protein, and cholesterol). Brewer et al. (2012) reported that studies which had been done to attempt with several feeding programmes using many dietary CP levels, showed that all the programs did not affect meat quality. The present study showed that the content of fat at Sensi-1 Agrinak

chicken meat was lower than the fat content of broiler chicken meat as reported by Hasanuddin et al. (2013), who showed that the fat content of broiler chickens meat was in the range of 4.88-7.38%. Contrastly, the content of cholesterol at Sensi-1 Agrinak chicken meat was higher than the content of cholesterol of broiler chickens meat,i.e., in the range of 0.046 – 0.049% (Abdulla et al. 2015)

CONCLUSION

Taking the performance, economic index aspect, meat bone ratio, and meat chemical composition traits of Sensi-1 Agrinak chicken into account, it is concluded that optimal dietary levels of crude protein and metabolizable energy for Sensi-1 Agrinak chicken was 21% CP and 3000 kcal ME/kg feed from 0-10 weeks of age.

REFERENCES

Abdulla NR, Loh TC, Akit H, Sazili AQ, Foo HL, Mohamad R, Abdul Rahim R, Ebrahimi M, Sabow. 2015. Fatty acid profile, cholesterol and oxidative status in broiler chicken breast muscle fed different dietary oil sources and calcium levels. South Afr J Anim Sci. 45:153-163.

Banerjee S, Melesse A, Dotamo E, berihun K, Beyan M. 2013. Effect of feeding different dietary protein levels with iso-caloric ration on nutrients intake and growth performances of dual-purpose Koekoeck chicken breeds. Int J Appl Poult Res. 2:27-32.

Bogosavljevic-Boskovic S, Mitrovic S, Djokovic R, Doskovic V, Djermanovic V. 2010. Chemical composition of chicken meat produced in extensive indoor and free range rearing systems. Afr J Biotechnol. 27:9069-9075.

Brewer VB, Emmert JL, Meullenet JFC, Owens CM. 2012. Small bird programmes: Effect of phase-feeding, strain, sex, and debone time on meat quality of broilers. Poult Sci. 91:499-504.

Chen JL, Zhao GP, Zheng MQ, Wen J, Yang N. 2008. Estimation of genetic parametres for contents of intra muscular fat and inosine -5 –monophospate and carcass traits in Chinese Beijing –You chickens. Poult Sci. 87:1098-1104.

Dewi GAMK, Mahardika IG, Sumadi IK, Suasta IM, Wirapartha M, Henuk YL. 2015. Effect of dietary energy and protein level on growth performance of native chickens at the starter phase. Khon Kaen Agr J. 43:216-210.

Diaz O, Rodriguez L, Torres A, Cobos A. 2010. Chemical composition and physico-chemical properties of meat from capons as affected by breed and age. Span J Agric Res. 8:91-99.

Hafiz AO, Hassan Z, Manap MNA. 2015. Effect of slaughtering methods on meat quality indicators, chemical changes and microbiological quality of broiler chicken meat during refrigerated storage. IOSR J Agric Vet Sci. 8:12-17

Hasanuddin S, Yunianto VD, Tristiarti. 2013. Lemak dan kolesterol daging pada ayam broiler yang diberi pakan step down protein dengan penambahan air perasan jeruk nipis sebagai acidifier. Buletin Nutrisi dan Makanan Ternak. 9:47-53.

Hasnelly, Iskandar S, Sartika T. 2017. Karakteristik kualitatif dan kuantitatif ayam SenSi-1 Agrinak. JITV. 22:68-79.

Horniakova E, Abas KA. 2009. Influence of low levels of protein and sex on carcass traits and nutrient content in broiler meats. Slovak J Anim Sci. 42:75-78.

Hidayat C, Iskandar S, Sartika T , Wardhani T. 2016. Growth response of improved native breeds of chicken to diets differed in energy and protein content. JITV. 21:174-181.

Liu SK, Niu ZY, Min YN, Wang ZP, zhang J, He ZF, Li HL, Sun TT, Liu FZ. 2015. Effects of dietary crude protein on the growth performance, carcass characteristics and serum biochemical indexes of Lueyang Black-boned chickens from seven to twelve weeks of age. Braz J Poult Sci. 17:103-108.

Magala H, Kugonza DR, Kwizera H, Kyarisiima CC. 2012. Influence of varying dietary energy and protein on growth and carcass characteristics of Ugandan local chickens. J Anim Prod Adv. 2:316-324.

Maliwan P, Khempaka S, Molee W. 2017. Evaluation of various feeding programmes on growth performance, carcass and meat qualities of Thai indigenous crossbred chickens. South Afr J Anim Sci. 47:16-25.

Marcu A, Vacaru-Opriş I, Marcu A, Nicula M, Dumitrescu G, Nichita I, Dronca D, Kelciov B. 2013. Effect of diets with different energy and protein levels on breast muscle characteristics of broiler chickens. Scientific Papers: Animal Science and Biotechnologies. 46:333-340.

Marcu A, Vacaru-Opriş I, Marcu A, Nicula M, Dumitrescu G, Nichita I, Dronca D, Kelciov B. 2011a. The influence of feed protein and energy level on the meat chemical composition at “Lohmann Meat” hybrid. Scientific Papers: Animal Science and Biotechnology. 44:439-443.

Marcu A, Vacaru-Opriş I, Marcu A, Nicula M, Dumitrescu G, Nichita I, Dronca D, Kelciov B. 2011b. The influence of feed energy and protein level on meat composition at “Arbor Acres” hybrid. [accessed February 17th 2019]. http://www.uaiasi.ro/zootehnie/Pdf/Pdf_Vol_56/Adela_Marcu.pdf.

Marcu A, Vacaru-Opriş I, Marcu A. 2009. The influence of feed protein and energy level on meat chemical composition from different anatomical regions at “Cobb 500” hybrid. Zootehnie şi Biotehnologii. 42:147-150.

Marcu A, Vacaru-Opriş I, Marcu A, Nicula M, Dumitrescu G, Nichita I, Dronca D, Kelciov B. 2012. The influence of protein and energy levelon meat chemical composition

at „Hybro PN+" broiler chickens. Scientific Papers Animal Science. USAMV Iaşi. 57:260-265.

Nguyen TV, Bunchasak C, Chantsavang S. 2010. Effects of dietary protein and energy on growth performance and carcass characteristics of Betong chickens *(Gallus domesticus)* during growing period. Int J Poult Sci. 9:468-472

Niu Z, Shi J, Liu F, Wang X, Gao C, Yao L. 2009. Effects of dietary energy and protein on growth performance and carcass quality of broilers during starter phase. Int J Poult Sci. 8:508-511.

[NRC] Nutrient Requirements of Poultry. 1994. 9th ed. Washington DC (USA): National Academy Press. ISBN-13: 9780309048927

Raphulu T, Rensburg CJV. 2018. Dietary protein and energy requirements of Venda village chickens. J Agric Rural Develop Trop Subtrop. 119:95-104.

Zaman QU, Mushtaaq T, Nawaza H, Mirza MA, Mahmood S, Ahmad T, Babar ME, Mushtaq MMH. 2008. Effect of varying dietary energy and protein on broiler performance in hot climate. Anim Feed Sci Tech. 146:302-312.

Phenotypic Characteristics of Exotic-Broiler, Kampung, Male Exotic-Layer, KUB-1 and Pelung Chickens

Saragih HTS[1], Viniwidihastuti F[2], Lembayu RP[2], Kinanthi AR[2], Kurnianto H[3], Lesmana I[1]

[1]*Staff Lecturer of Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, 55281*
[2]*Student of Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, 55281*
[3]*Central Java Assessment Institute for Agricultural Technology, IAARD, Ministry of Agriculture*
E-mail: saragihendry@ugm.ac.id

(received 25-10-2018; revised 24-01-2019; accepted 24-01-2019)

ABSTRAK

Saragih HTS, Viniwidihastuti F, Lembayu RP, Kinanthi AR, Kurnianto H, Lesmana I. 2019. Karakteristik fenotip ayam broiler eksotik, kampung, layer eksotik jantan, KUB-1 dan Pelung. JITV 24(1): 9-14. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1889

Kebutuhan daging ayam selama ini didominasi oleh daging ayam yang berasal dari jenis ayam broiler eksotik (tetuanya eks impor). Potensi ayam pedaging lokal di Indonesia diharapkan mampu menyediakan ketersediaan kebutuhan daging ayam nasional. Tujuan dari penelitian ini adalah untuk mengetahui karakteristik fenotip *day old chick* (DOC) jenis-jenis ayam yang ada di Indonesia yang memiliki potensi sebagai ayam pedaging. Karakter fenotip yang dilihat berupa morfometri tubuh, berat organ viseral dan morfologi usus halus. Ayam yang digunakan merupakan DOC jenis ayam broiler eksotik, Kampung, Layer eksotik jantan, KUB-1 dan Pelung. Sebanyak 125 ekor yang terdiri dari 25 ekor setiap *strain* ayam pada umur *posthatched* diamati morfometri tubuhnya, berat organ viseral dan histo-morfologi usus halusnya. Data pengamatan kemudian dianalisa menggunakan analisis similaritas dengan menggunakan MVSP 3.22 untuk membuat dendogram dengan metode UPGMA (*Unweighted Pair Group with Aritmetic Average*). Hasil penelitian menunjukkan bahwa terdapat 2 klaster berbeda terhadap tingkat kesamaan dalam karakternya. Ayam broiler memiliki persentase similaritas 94,65% terhadap Ayam layer jantan dan 92,26 % terhadap ayam pelung. Sementara ayam Kampung memiliki persentase similaritas 90,16% terhadap Ayam KUB-1. Kesimpulan penelitian ini menunjukkan bahwa tingkat kesamaan karakter fenotip ayam pelung mendekati jenis ayam broiler dan jantan layer. Tingkat kesamaan ini diharapkan bahwa ayam pelung dapat sebagai jenis ayam lokal yang berpotensi sebagai ayam pedaging.

Kata Kunci: *Day Old Chick*, Morfometri, Organ

ABSTRACT

Saragih HTS, Viniwidihastuti F, Lembayu RP, Kinanthi AR, Kurnianto H, Lesmana I. 2019. Phenotypic characteristics of Exotic-broiler, Kampung, Male Exotic-layer, KUB-1 and Pelung chickens. JITV 24(1): 9-14. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1889

The needs for chicken meat have been dominated by meat from exotic broilers (bred from imported parent). The potential of local broilers chicken in Indonesia is expected to be able to provide the meat for national needs for chicken meat. The objective of this research was to determine the day-old-chick (DOC) phenotypic characteristics of chickens in Indonesia that have the potential as broilers. The phenotypic characteristics are in the form of body morphometry, visceral organ weight and small intestinal histo-morphology. The chickens used were the DOC type of exotic Broiler, Kampung, exotic male Layer, KUB-1 and Pelung. A total of 125 chickens consisting of 25 chickens of each strain at posthatched age were observed for its body morphometry and its visceral organ weight, and small intestinal histo-morphology. The observed data were then analyzed by similarity analysis using MVSP 3.22 to create a dendogram with the Unweighted Pair Group with Aritmetic Average (UPGMA) method. Results showed that there were 2 different clusters from the level of similarity in their characteristics. Exotic broilers had 94.65% similarity to male exotic layer chicken and 92.26% to pelung chicken, while Kampung chickens had 90.16% similarity to KUB-1Chicken. In conclusion, it is indicated that the level of similarity of the phenotypic characteristics of pelung chickens were close to the type of exotic broiler and male exotic layer chicken. This level of similarity lead to the expectation that pelung chickens potential to be a candidate for meat-type of local chicken.

Key Words: Day Old Chick, Morphometry, Organs

INTRODUCTION

Food proteins source derived from animal such as beef, sheep, goat, and chicken meats are in great demand for Indonesian people. Protein needs through chicken meat have been mostly fulfilled by exotic broiler chickens. This dominance is very reasonable because the exotic broiler chicken grow faster, which is within 35 days they are ready to be harvested with an average live weight of 2 kg per chicken. Besides the fast growth, the availability of broiler chicken meat has also been supported by big modern broiler chicken companies. (Tamalludidi 2012).

The attempts to find alternative chicken meat availability apart from broiler chickens continue to be carried out by the Indonesian government and the

society. Indonesian government through The Indonesian Research Institute for Animal Production (IRIAP) has been continuing research. to create innovations in meeting the demand of . local chicken DOC. Moreover the society has also been trying to increase local chicken meat by crossing male local chickens with exotic laying hens. The product are commonly called as Jawa Super chickens (Setiawan et al. 2018).

The pure local breed of chickens that have high potential of body weight is Pelung (Nataamijaya 2000). Pelung chicken, besides being known as ornamental chicken that has long crowing sounds, it also has a good growth ability to reach body weight of 1.1-1.3 kg in 3 months (Iskandar & Susanti 2007).

Local chicken has the potential to become a national chicken meat industry because so far the society as consumers prefer local or kampung chicken meat. Kampung chicken meat more recognizable by Indonesian consumers as it is more delicious taste compared to exotic broiler chicken meat. However, the frequently-faced problems by local chicken producers was the low productivity of kampung chickens either in body growth of only optimally of 1.2-1.5 kg/ chicken or egg weight which ranged between 30-40 grams (Nataamijaya 2010). Recently, there are improved broiler type of local chicken, called SenSi-1 Aagrinak (Iskandar 2018) and improved egg type of local chicken called KUB-1 chicken (Sartika et al. 2013). Both breeds were produced at the IRIAP and disseminated throughout the country through licencing system to private local chicken breeder companies (Iskandar 2018).

Knowledge of the phenotypic characteristics of the types of chicken in Indonesia is very important to see the potential of broilers in Indonesia. Therefore, research needs to be conducted since the age of DOC in order to get information from the beginning of the phenotypic characteristics of these types of chicken.

Therefore, this research was aimed to determine the phenotypic characteristics of several breeds of chickens in Indonesia which have the potential to become broiler type of chickens in order to meet the needs of the National chicken meat.

MATERIALS AND METHODS

The research was carried out in stages, which are the measurements of chicken morphometry, visceral organ and villus height, crypt depth and number of small intestine goblet cells (duodenum, jejunum and ileum). The entire research was conducted at the Laboratory of Animal Structure and Development of the Faculty of Biology, Gadjah Mada University.

In this research used 125 DOC were used, nconsisting of 25 chickens from each breed or strain. There were exotic (which was a modern breed derived from imported parent or grand parent) broiler, kampung, exotic male layer, Kampung KUB and Pelung chickens. The chicks of exotic broiler, kampung, pelung and exotic male layer chickens were obtained from chicken distributors in Yogyakarta area, while KUB-1 chickens were obtained from the farmer partners of IRIAP in Berbah, Tirtosari, and Yogyakarta regions. Observations of the phenotypic characteristics included the following steps:

Measurement of chicken morphometric

Morphometric measurement was performed on DOC of all 5 breeds of chickens. The measurement conducted refered to the chicken phenotypic characteristics using midline and ruler.

Measurement of chickens visceral organ weight

Measurement of visceral organ weight was performed on DOC of all 5 breeds of chicken. The measurement was carried out on 10 chicks from each breeds using semi-analytic scales. The measurements of chickens' visceral organ weight included the liver, heart, proventriculus, ventriculus, length of the small intestine, weight of the small intestine, weight of the bursa of fabricius, weight and area of 1/2 part of the *Pectoralis thoracicus* muscle (PT).

Preparation of small intestine histology slides

Ten DOCs from each breed were sacrificed. The chickens were then dissected and duodenum, jejunum and ileum, were taken.The organs taken were then fixed in Bouin solution for approximately 12 hours. Afterwards, the organs were washed using 70% alcohol until the solution was not yellow.

After washing the organs were dehydrated. Dehydration of the small intestine was done using multilevel alcohol and then proceed with the process of clearing and infiltration. The embedding was performed using pure liquid paraffin. The next stage was trimming and sectioning which was followed by Hematoxylin & Eosin (H&E) and Periodic Acid Schiff's (PAS) staining.

The stages of measurement of villus height, crypt depth and number of small intestine goblet cells were carried out by finding the location of the villus and crypt, which was observed under a light microscope with a magnification of 4 x 10 and 10 x 10. The observation results were photographed using a Leica microscope, connected to a computer, and analyzed using raster image and J. Imagesoftware.

Data analysis

The measurement of research data used were semi analytic scales, midline scale, Leica microscopy, ImageJ program, Image Raster 3. The data obtained were quantitative data of chickens' morphometry, visceral organ weight, villus height and crypt depth, ratio of villus height/crypt depth, goblet cell area and number of goblet in 100 µm villus height. The data

were analyzed using SPSS 13.0 and the similarity analysis using MVSP 3.22 to create a dendogram with the UPGMA method (Unweighted Pair Group with Aritmetic Average).

RESULTS AND DISCUSSION

The characteristics of morphometry (Table 1), visceral organ (Table 2), and small intestine structure (Table 3 and Figure 1) on DOC of the 5 breeds observed, were analysed using UPGMA method (Unweighted Pair Group with Aritmetic Average), showing that there were 2 clusters of similarity level. UPGMA analysis result was in the form of dendogram that showed the similarity level of characteristics in the type of chicken used (Figure 2). The five types of chicken used in this research had a similarity level of 86.39%. The first cluster, which was kampung and KUB-1 chickens, has the highest similarity level reaching 90.16%. The second cluster consisted of Pelung, exotic male layer and exotic broiler chickens with a similarity level of 92.26%. This second cluster formed two different clusters, Pelung Chicken, that formed a separate cluster from exotic male layer and exotic broiler chickens. The results also showed that the similarity level between exotic male layer and exotic broiler chickens was 94.65%.

The analysis results of the phenotypic characteristics and similarity levels indicated that exotic broiler chickens had better phenotypic characteristics -for-meat type. Exotic broilers had been known as the type of chickens for meat having fast body growth. (Tamalludidi 2012) reported that exotic broiler chicken was usually ready to be harvested at the age of 35 days with a maximum body weight of 2-3 kg. Chicken growth was believed to be influenced by genetic and nutritional and management factors (Tallentire et al. 2016; Neves et al. 2014). Tallentire et al. (2016) reported that a good genetic selection process followed by balanced nutrition in broiler chickens could provide efficient breeding process.

Results of this research also showed that exotic male layer chickens had quite strong similarity number of caharacteristics to exotic broiler chickens. Zaheer (2015) explained that domestication of modern chicken from members of the Gallus genus probably 8 thousand years ago in the Southern Asia. Exotic layer chicken was a type of modern chicken that was intended as laying hens and during this time exotic male layer chickens were not optimized. Currently in Indonesia it was being used as an alternative to exotic broilers due to its fairly good growth compared to local chickens. Exotic male layer chickens were recognized to reach 1.18 kg in 6 weeks (Daud et al. 2017).

Table 1. Analysis of morphometry of day old chick (DOC) of exotic broiler, kampung, exotic male layer, KUB-1 and Pelung chicken breeds

Characteristic	Exotic broiler	Kampung	Exotic male layer	KUB-1	Pelung
Body weight, (gr)	41.32±1.04	26.20±3.39	38.36±1.86	28.67±1.73	35.58±3.47
Total height, (cm)	12.00±0.38	7.50±0.47	11.30±0.64	10.33±0.43	10.75±0.52
Body height, (cm)	8.56±0.49	5.79±0.33	8.26±0.35	7.73±0.34	8.42±0.66
Beak width, (cm)	1.03±0.21	0.91±0.09	1.31±0.11	1.17±0.1.16	1.12±0.04
Beak length, (cm)	1.28±0.10	1.19±0.13	1.46±0.05	1.28±0.08	1.27±0.05
Head length, (cm)	4.14±0.31	1.97±0.09	3.99±0.11	3.63±0.22	3.62±0.28
Head width, (cm)	2.38±0.33	1.69±0.23	2.27±0.21	1.87±0.13	1.82±0.12
Body length, (cm)	8.31±0.37	4.32±0.30	8.92±0.24	7.92±0.29	9.23±0.59
Body width, (cm)	4.50±0.38	4.08±0.37	4.27±0.25	3.80±0.18	4.23±0.23
Chest circumference, (cm)	10.88±0.83	7.55±0.54	10.58±0.44	10.00±0.40	10.94±0.75
Wing length, (cm)	5.44±0.39	4.05±0.18	4.51±0.20	3.78±0.37	4.86±0.35
Neck length, (cm)	2.44±0.49	2.31±0.44	2.47±0.14	2.55±0.26	3.43±0.79
Thigh length, (cm)	3.86±0.23	3.17±0.23	3.86±0.14	3.73±0.30	3.59±0.16
Calves length, (cm)	3.83±0.26	3.31±0.22	3.67±0.25	3.69±0.19	3.86±0.32
Tibia tarsus, (cm)	4.95±0.31	4.32±0.17	4.87±0.14	4.71±0.23	4.91±0.18

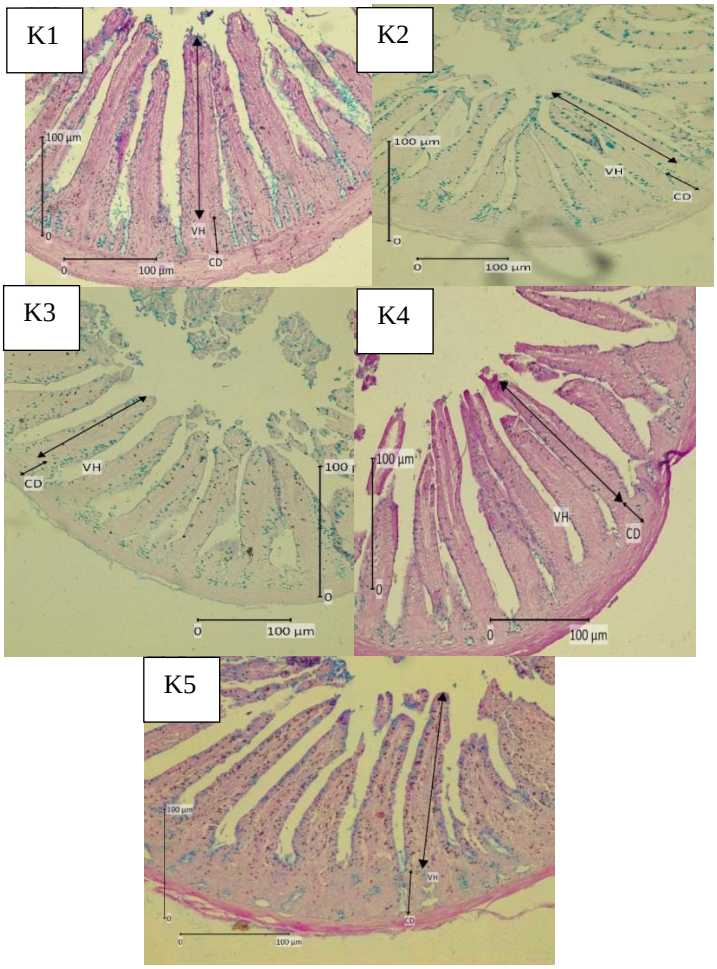


Figure 1. Duodenum morphology of DOC with 10x10 magnification and PAS stain. VH: Villus Height. CD: Crypte Depth.K1: Exotic broiler chicks; K2: Kampung chicks; K3: Exotic male layer chicks; K4: KUB-1 chicks. K5: Pelung chicks.

The current research also looks at the similarity values of Pelung, Kampung and KUB-1 chickens which were the types of chicken developed by IRIAP. KUB-1 chicken was the result of selection of kampung chickens. According to Sartika et al. (2013), KUB-1 chicken were black feather color, striated color, columbian type, and white feather color. The adult male body weight ranges from 1.3-1.6 kg while in females it ranges from 1.2-1.4 kg at peak production. Kampung chickens had slow growth and little egg production capabilities. Kampung chickens could achieve optimal growth of around 1.2-1.5 kg (Nataamijaya 2010). Pelung chicken is a native Indonesian chicken that has been developed in Cianjur area, West Java, Indonesia. Nataamijaya (2010) reported that pelung chickens, besides having superiority in crowing, it could also be used as broiler type of chicken. Pelung chicken in 90 days old had ability to reach body weight of 1.1-1.3 kg (Iskandar & Susanti 2007).

This research also reported body morphometric values, visceral organ weight and small intestinal histo-morphology among all types of chickens observed. Results showed that morphometric values, visceral organ weight and small intestinal morphology of exotic broiler DOC were still better than DOC group of other types of chicken. This result is in line with the research conducted by Kokoszyński et al. (2017) which explains that broilers have superior body conformation to support growth. The advantage of morphometric measurement method is that morphometry provides a description of quantitative elements. Morphometric data analysis could find out the part where the important changes occur as the references for the research to be (Zelditch et al. 2004). Some of the most important quantitative characteristics in bird morphometry were body weight, femur length, tarsometatarsus, circumference of tarsometatarsus, third finger length and wings (Kurniawan & Arifianto 2017).

Visceral organs of chickens such as the heart, liver and digestive system are important parts of metabolic process of the body. The heart organ has the ability to circulate blood to the lungs in the exchanging process of O_2 and CO_2 to help the metabolic process of the body. The liver organ functions to secrete bile to be channeled to the duodenum. Bile can neutralize

Table 2. Analysis visceral organ DOC of exotic broiler, kampung, exotic male layer, KUB-1 and Pelung chicken breeds

Visceral organ	Exotic broiler	Kampung	Exotic male layer	KUB-1	Pelung
Liver, (gr)	1.54±0.13	1.15±0.33	1.12±0.07	0.94±0.09	1.29±0.05
Heart, (gr)	0.44±0.05	0.26±0.04	0.33±0.05	0.25±0.03	0.25±0.02
Ventriculus, (gr)	0.48±0.04	0.33±0.07	0.45±0.03	0.31±0.03	0.48±0.03
Proventriculus, (gr)	3.06±0.33	2.25±0.39	3.03±0.15	1.85±0.22	2.81±0.24
Small intestine length, (cm)	43.49±2.73	33.37±3.85	45.06±3.43	33.83±2.41	57.38±2.49
Small intestine weight, (gr)	1.78±0.09	1.13±0.21	1.64±0.07	0.84±0.09	2.95±0.45
Bursa fabricius weight, (gr)	0.064±0.02	0.043±0.02	0.063±0.02	0.044±0.01	0.09±0.05
1/2 Pectoralis muscle weight, (gr)	0.96±0.08	0.47±0.07	0.83±0.06	0.66±0.08	1.07±0.03
1/2 Pectoralis muscle area, (cm^2)	4.97±0.33	3.29±0.29	4.12±0.44	3.80±0.32	5.86±0.20

Table 3. Analysis of villus height, crypte depth, villus/crypte ratio, number of Goblet cells and area of Goblet cell DOC of exotic broiler, kampung, exotic male layer, KUB-1 and Pelung chicken breeds

Variable / Duodenum	Exotic broiler	Kampung	Exotic male layer	KUB-1	Pelung
Villus height, (µm)	191.32±9.98	142.12±6.87	178.67 ±6.75	161.92±8.66	235.63±5.19
Crypte depth, (µm)	45.94±3.54	32.97±2.62	31.99±1.79	37.43±2.38	46.04±7.61
V/Cratio	4.12±0.27	4.51±0.35	5.59±0.29	4.34±0.29	5.22±0.80
Number of Goblet cells/ 100	30.20±1.48	29.00±1.22	30.60±2.30	25.60±5.59	26.5±1.91
Area of Goblet cell, (μm^2)	10.50±1.02	7.81±1.06	9.32±1.16	5.96±0.59	9.65±1.35
Jenjunum					
Villus height, (µm)	144.92±5.05	126.3±6.07	124.99 ±7.81	78.78±3.46	138.54±2.66
Crypte depth, (µm)	30.89±2.56	25.46±3.01	27.61±1.04	19.24±1.53	40.89±2.36
V/Cratio	4.72±0.47	5.02±0.49	4.53±0.31	4.11±0.31	3.39±0.23
Number of Goblet cells/ 100	34.20±3.42	43.00±7.48	38.20±5.17	31.20±4.15	32.40±5.46
Area of Goblet cell, (μm^2)	8.98±1.32	7.53±1.27	8.67±0.61	4.89±0.54	8.13±1.49
Ileum					
Villus height, (µm)	125.93±4.15	95.40±7.49	138.01 ±6.75	102.02±9.42	137.73±7.69
Crypte depth, (µm)	34.33±5.44	27.47±1.25	27.47±1.25	23.55±1.41	38.59±6.77
V/Cratio	3.68±0.24	3.8±0.75	5.04±0.43	4.33±0.26	3.63±0.49
Number of Goblet cells/ 100	30.20±2.68	24.60±2.30	29.60±4.22	28.80±1.79	37.20±2.77
Area of Goblet cell, (μm^2)	13.14±1.75	10.60±0.80	11.27±1.46	10.04±0.76	13.29±0.94

stomach acid and form an emulsion in the stomach. Both functions help absorption and translocation of fatty acids during the absorption of feed essence (Swatson et al. 2003). (Kokoszyński et al. 2017) reported that broilers are known to have the ability in weight growth which is supported by the balance of its visceral organs.

The digestive part in the chicken's body is an important part in the absorption process of nutrients. The small intestine consisting of the duodenum, jejunum and ileum is the essential part for such process. The small intestinal morphology such as villous height, crypt depth and number of goblet cells plays an important role in increasing the absorption ability, preventing the occurrence of inflammation and preparing for the regeneration of epithelial cell (Rajput et al. 2013; Uni et al. 2003; Deplancke & Gaskins 2001). Broilers are known to have good small intestinal morphology for the process of absorption in the intestine and in line with the growth of body weight (Ibrahim 2008).

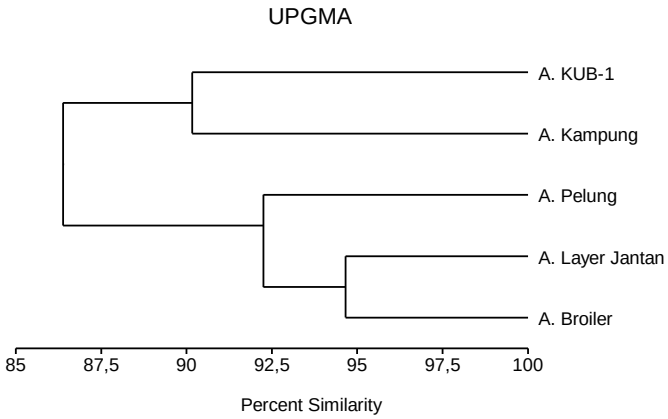


Figure 2. Percentage similarity of dendogram morphometry, visceral organ and small intestine morphology on day old chick (DOC) of exotic broiler, kampung, exotic male layer, KUB-1 and Pelung chicken breeds using UPGMA methods.

CONCLUSION

The similarity level of pelung chicken as Indonesian local chicken was quite close to the type of exotic broiler and exotic male layer chicken so it was promising as an alternative for meat type of local chicken.

REFERENCES

Daud M, Fuadi Z, Mulyadi M. 2017. Performan dan persentase karkas ayam ras petelur jantan pada kepadatan kandang yang berbeda. J Agripet. 17:67.

Deplancke B, Gaskins HR. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr. 73:1131S-1141S.

Ibrahim S. 2008. Hubungan ukuran-ukuran usus halus dengan berat badan broiler. J Agripet. 8:42.

Iskandar S. 2018. Phenotypic characterization and distribution of Sensi-1 Agrinak chicken. Wartazoa. 28:51-60.

Iskandar S, Susanti T. 2007. Karakter dan manfaat ayam pelung di Indonesia. Wartazoa. 17:128-136.

Kokoszyński D, Bernacki Z, Saleh M, Stęczny K, Binkowska M. 2017. Body conformation and internal organs characteristics of different commercial broiler lines. Rev Bras Ciência Avícola. 19:47-52.

Kurniawan N, Arifianto A. 2017. Ornitologi: Sejarah, biologi dan konservasi. Malang (Indones): UB Press.

Nataamijaya AG. 2000. The native chickens of Indonesia. Bul Plasma Nutfah. 6:1-6.

Nataamijaya AG. 2010. Pengembangan potensi ayam lokal untuk menunjang peningkatan kesejahteraan petani. J Penelitian Pengembangan. 29:131-138.

Neves D, Banhazi T, Nääs I. 2014. Feeding behaviour of broiler chickens: a review on the biomechanical characteristics. Rev Bras Ciência Avícola. 16:01-16.

Rajput N, Muhammad N, Yan R, Zhong X, Wang T. 2013. Effect of dietary supplementation of curcumin on growth performance, intestinal morphology and nutrients utilization of broiler chicks. J Poult Sci. 50:44-52.

Sartika T, Desmayanti, Iskandar S, Resnawati H. 2013. Ayam KUB-1. Jakarta (Indones): IAARD Press.

Setiawan H, Jingga ME, Saragih HT. 2018. The effect of cashew leaf extract on small intestine morphology and growth performance of Jawa Super chicken. Vet World. 11:1047-1054.

Swatson HK, Iji PA, Gous RM. 2003. Body growth, visceral organ weight and intestinal digestive enzyme fchickens on diets varying in energy and protein contents. J Anim Vet Adv. 2:305-311.

Tallentire CW, Leinonen I, Kyriazakis I. 2016. Breeding for efficiency in the broiler chicken: A review. Agron Sustain Dev. 36:66.

Tamalludidi F. 2012. Ayam broiler, 22 hari panen lebih untung. Jakarta (Indones): Penebar Swadaya.

Uni Z, Smirnov A, Sklan D. 2003. Pre- and posthatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. Poult Sci. 82:320-327.

Zaheer K. 2015. An updated review on chicken eggs: production, consumption, management aspects and nutritional benefits to human health. Food Nutr Sci. 06:1208-1220.

Zelditch ML, Swiderski DL, Sheetsy HD, Fink WL. 2004. Geometric morphometrics for biologists: A primer. Rio de Janeiro (Braz): Elsevier Ltd.

Effect of Addition of Selected Amino Acids in Semen Extender on Quality and DNA Stability of Frozen-Thawed Sumba Ongole Bull Spermatozoa

Said S[1], Setiorini[2], Adella M[2], Sari I[2], Fathaniah N[2], Maulana T[1]

[1]*Research Center for Biotechnology, Indonesian Institute of Sciences, Jln. Raya Bogor Km.46 Cibinong 16911.*
[2]*Department of Biology, Faculty of Mathematics and Natural Science, University of Indonesia. Kampus UI Depok 16424.*
E-mail: syahruddinsaid01@gmail.com

(received 05-10-2018; revised 18-01-2019; accepted 24-01-2019)

ABSTRAK

Said S, Setiorini, Adella M, Sari I, Fathaniah N, Maulana T. 2019 Pengaruh penambahan asam amino terseleksi pada pengencer semen terhadap kualitas dan stabilitas DNA sperma beku sapi Sumba Ongole. JITV 24(1): 15-21. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1873

Penelitian ini bertujuan mengetahui konsentrasi optimal asam amino glutamin, glisin dan sistein dalam *tris-citric-acid-fructose egg yolks* (TCFY) terhadap kualitas spermatozoa sapi Sumba Ongole (SO) selama proses pembekuan dan *thawing*. Penelitian ini juga mengidentifikasi stabilitas DNA sperma beku. Tiga ekor pejantan dewasa digunakan sebagai donor semen yang dipelihara di PT. Karya Anugerah Rumpin, perusahaan pembibitan sapi swasta, Jawa Barat, Indonesia. Semen dikoleksi menggunakan vagina buatan, dievaluasi sebelum dibekukan. Semen diencerkan dengan TCFY setelah penambahan masing-masing asam amino dengan konsentrasi berbeda (5, 15 dan 25 mM glisin dan glutamin, dan 3, 5 dan 7 mM sistein) selanjutnya di equilibrasi dan dibekukan. Parameter kualitas sperma yang diamati adalah motilitas, viabilitas, membran plasma utuh, dan stabilitas DNA). Data yang diperoleh memperlihatkan bahwa secara umum pengaruh penambahan asam amino terseleksi (glisin, glutamin dan sistein) kedalam ekstender TCFY terhadap motilitas, viabilitas dan membran plasma utuh sperma sapi SO setelah equilibrasi signifikan (P<0,05) berbeda lebih baik daripada kontrol. Penambahan 15 mM glisin, 15 mM glutamin dan 5 mM sistein signifikan (P<0,05) meningkatkan motilitas, viabilitas dan membran plasma utuh sperma setelah *thawing* dibandingkan *control*. Ketika sperma difiksasi dengan asam alkohol, diwarnai dengan *acridine orange*, menunjukkan bahwa seluruh spermatozoa yang diamati DNA nya tetap stabil. Disimpulkan bahwa penambahan 15 mM glisin, glutamin dan 5 mM sistein meningkatkan kemampuan memelihara sperma dalam kondisi beku, dan DNA sperma menunjukkan tetap stabil.

Kata Kunci: Kriopreservasi, Semen Sapi, Glisin, Glutamin, Sistein

ABSTRACT

Said S, Setiorini, Adella M, Sari I, Fathaniah N, Maulana T. 2019. The effect of addition selected amino acids in extender semen on quality and DNA stability of frozen-thawed Sumba Ongole bull spermatozoa. JITV 24(1): 15-21. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1873

The objective of the current study was to asses the optimal concentration of glutamine, glycine and cysteine amino acids in tris-citric-acid-fructose egg yolks (TCFY) extender on quality of SO bull spermatozoa during freezing and thawing. In this study the DNA stability of frozen-thawed Sperm was also indentified. Three mature bulls maintained at PT. Karya Anugerah Rumpin, private cattle breeding company, West Java, Indonesia were used as semen donors. Semen was collected using artificial vagina and were evaluated prior to freezing. Semen was diluted with TCFY supplemented with different concentrations of amino acids (5, 15 and 25 mM glycine and glutamine, and 3, 5 and 7 mM cysteine) then processed for colling and freezing. Semen quality parameters (subjective motility, viability and membrane and DNA integrity). Data showed that in general the effect of addition of selected amino acids (glycine, glutamine and cysteine) into TCFY extenders on motility, viability and membrane integrity of SO spermatozoa after cooling were significantly different (P<0.05) higher than that of control. Addition of 15 mM glycine, 15 mM glutamine and 5 mM cysteine resulted in significant (P<0.05) increase post-thawing sperm motility and sperm viability as compared to that of control. Furthermore, when spermatozoa were stained with acridine orange after fixation with acetic alcohol, the DNA integrity of post-thawing spermatozoa showed that all spermatozoa were remain intact. In conclusion ,addition of 15 mM glycine, glutamine and 5 mM cysteine increase the cryoprotecting efficacy of bovine bull cryopreservation extender, and furthermore all DNA spermatozoa were remain intact.

Key Words: Cryopreservation, Bovine Semen, Glycine, Glutamine, Cysteine

INTRODUCTION

Sumba ongole (SO) (*Bos indicus*) cattle is one of Indonesia superior local cattle that plays an important role in meat production for society. It is necessary to improve the population and genetic quality of SO cattle by sperm cryopreservation and artificial insemination (AI). Through this technique, spermatozoa derived from

superior bulls can fertilize many cattles without any limitations of distance and time (Tsai & Lin 2012).

Cryopreservation of bull semen is still challenging due to lower fertility when compared to fresh semen because the all processes of cryopreservation including cooling, freezing and thawing create oxidative stress on the sperm membrane (Chatterjee et al. 2001). Freezing/thawing of sperm sample is routinely performed in cattle breeding industries in order to perform artificial insemination. During cryopreservation, semen is exposed to cold shock and atmospheric oxygen, which in turn increases the susceptibility to lipid peroxidation due to higher production of ROS (Bucak et al. 2008). Oxidative stress generally leads to loss of motility, swelling and the blebbing of the acrosomal membrane and disruption or increased permeability of the plasma membrane of spermatozoa (White 1993). It is well known that mammalian spermatozoa contain high concentrations of polyunsaturated fatty acids, and therefore are highly vulnerable to oxidative stress which is responsible for the generation of reactive oxygen species (ROS) (Cassani et al. 2005).

The improvement of cryopreservation technique requires in depth knowledge of the gamete physiology and the biochemical processes occurring during semen collection, processing, and freezing-thawing. Damage due to oxidative stress may be passed by the inclusion of antioxidants prior to freezing processes (Bucak et al. 2008). One of the natural antioxidants present in the seminal plasma is the glutahione (GSH) (Atig et al. 2012). Glutathione is amino acid that formed from glutamine, cysteine, and glycine. However, Glutathione (GSH) in seminal plasma may be reduced by the process of dilution and freezing in cryopreservation. This causes the spermatozoa to be more susceptible to oxidative stress due to the reduced antioxidant content in the plasma seminal (Kutluyer & Kocabas 2016).

Previous studies reported that the optimal concentrations of glutamine in cryopreservation extender were 25 mM in goat (Ali Al Ahmad et al. 2008), 10 mM in bull (Amirat et al. 2004), 50 mM in stallion (Khlifaouia et al. 2005) and in man (Renard et al. 1996). It was observed that addition of 25 mM of glutamine, 25 mM glycine and 5 mM cyctein increase the cryoprotecting efficacy of buffalo bull cryopreservation extender (El-Sheshtawy et al. 2008). There are no studies on the effect of selected amino acids addition into semen extender on quality of frozen-thawed Sumba Ongole bull as well known Indonesian local breed.

The main objective of the current study was to asses the optimal concentration of glutamine, glycine and cysteine amino acids in tris-citric-acid-fructose egg yolks (TCFY) extender on spermatozoa quality of SO bull during freezing and thawing. The stability of frozen-thawed Sperm DNA also indentified in this study.

MATERIALS AND METHODS

Animal

Three bulls maintained at PT. Karya Anugerah Rumpin, private cattle breeding company, West Java, Indonesia were used as semen donors. Semen was collected using artificial vagina during mid-morning after an extended period of routine collection every week. Semen quality consist of volume, concentration, motility and abnormality were evaluated prior to freezing. Ejaculates fulfilling minimum standard of sperm motility (70%) and sperm morphologically normal (80%) were used for this study.

Frozen-thawed spermatozoa

The reference cryopreservation extender as control was Tris-citric-acid-fructose egg yolk (TCFY) diluent, the medium containing Tris (hydroxymethyl) aminomethan (3.09%), citrate acid (1.73%), fructose (1.27%), and added with 20% (v/v) egg yolk and antibiotic 1% (v/v). The amino acids glutamine, glycine (Merck Chemical Co. Germany) were added to the control extender at concentration of 5 mM, 15 mM, and 25 mM, while cysteine (Merck Chemical Co. Germany) was added at concentration of 3 mM, 5 mM, and 7 mM. Semen was diluted with each extender to provide concentration of 25 million spermatozoa in a 0.25 ml polyvinyl straw. Packed semen in 0.25 ml straw was then equilibrated at 4°C for 2 hours, in that time, semen was evaluated. After equilibration periods, the straws were placed horizontally on a rack and frozen in a vapour 5 cm above liquid nitrogen for 10 minutes, and then dipped in liquid nitrogen (Said et al. 2015).

Assessment of semen quality parameters

The assessment of SO bull spermatozoa was undertaken on neat semen, equilibration period and freeze thawing. Frozen straws were thawed in waterbath at 37°C for 30 seconds. The subjective motility, viability, membrane integrity of spermatozoa performed after equilibration, and after thawing. In addition, evaluation of sperm DNA integrity was performed after thawing.

Sperm motility

Sperm motility (%) was assessed subjectively using microscope set at magnification of 200X. Semen sample was diluted 200 times in NaCl physiological.

The diluted semen is then dripped on the improved Neubauer chamber and observed. The number of immobilized spermatozoa was calculated first, then after all the spermatozoa did not move, the total sperm was calculated (Akcay et al. 2004).

Sperm viability

Sperm viability (%) was assessed using microscope set at magnification 200. Semen sample mixed with eosin 2%, then smeared on object glass. One hundred spermatozoa were assessed and the percentage of unstained (alive) spermatozoa were calculated (WHO 2010).

Sperm membrane integrity

Sperm membrane integrity (%) was determined by calculation of the percentage of spermatozoa having intact plasma membrane by osmotic resistance test method (Revell & Mrode 1994). Hypo-osmotic solution composition comprising: 0.9 g of fructose, 0.49 g of sodium citrate were dissolved with aquabidestilata up to a volume of 100 ml. A total of 200 µL of hypo-osmotic solution was added to 20 µL semen, mixed until homogeneous, then incubated at 37°C for 45 minutes. Semen samples were smeared on a glass object and evaluated with 400X magnification. One hundred spermatozoa were assesed and the percentage of spermatozoa with curled tail (swelling) were calculated.

Sperm DNA integrity

DNA integrity of frozen-thawed spermatozoa was evaluated by using acridine orange staining technique. Samples of spermatozoa were smeared on glass slides, air-dried, fixed for 2 h in acetic alcohol (1 part glacial acetic acid plus 3 parts 100% methanol) and air- dried again. After fixation, sperm samples were stained with acridine orange solution (at 1000x dilution with GL-PBS) overnight (Said et al. 2003). After staining, each slide was washed with distilled water and sealed with synthetic resin to prevent it from drying. Slides were examined with fluorescence microscope (Axiophot Zeiss; 490/530 nm excitation/barrier filter). Two hundred cells were analyzed in each treatment slide. Sperm with normal DNA content presented a green fluorescence, whereas sperm with abnormal DNA content emited fluorescence in a spectrum varying from yellow to green. The percentage of sperm with intact chromatin was calculated by dividing the number of green-stained sperm by the total number of sperm and multiplying the result by 200 (Said et al. 1999).

Statistical analysis

The data were analyzed using Statistical Product and Service Solution (SPSS) version 24. Data normality was tested using Shapiro & Wilk test, then homogenity was tested using Levene test. The data were normally distributed and homogeneously varied, so the test continued using a one-factor variance analysis (ANOVA) test and continued with the Tukey test to see whether there were differences between treatments (Zar 1974).

RESULTS AND DISCUSSION

Quality of frozen-thawed Sumba Ongole bull spermatozoa

The effect of addition selected of amino acids (glycine, glutamine and cysteine) to TCFY extenders used for processing of SO bulls semen on motility, viability, membrane integrity after equilibration period summarized in Table 1.

These data showed that in general the effect of addition of selected amino acids (glycine, glutamine and cysteine) to TCFY extenders on motility, viability and membrane integrity of SO spermatozoa after equilibration were significantly ($P<0.05$) higher than that of control. Except in cysteine 3 mM and 7 mM the effect on viability and membrane integrity sperm were not significantly different ($P>0.05$) than control. The data also indicated that within each amino acid, additions of 5 mM glycine and 15 mM glutamine enhance sperm motility, whereas 5 mM cysteine enhance sperm motility, viability and membrane integrity during equilibration.

The effect of addition of selected amino acids (glycine, glutamine and cysteine) to TCFY extenders used for processing of SO bulls semen on motility, viability, membrane integrity post-thawing summarized in Table 2.

Data in Table 2 shows that addition of 15 mM glycine, 15 mM glutamine and 5 mM cysteine resulted in significant ($P<0.05$) increase post-thawing sperm motility and sperm viability as compared to the control. However, addition of selected amino acids to the extender were not significantly different on membrane integrity with no further significant enhancement of sperm motility, viability could be noticed when glycine and glutamine were added at concentration of 15 mM and cysteine of 5 mM.

The cryopreservation of semen includes the decrease in temperature and increase in oxidative stress

Table 1. Effect of different concentrations of selected amino acids on characteristics of SO bull spermatozoa after equilibration (Mean ± SE)

Treatment		Semen characteristics		
		Motility	Viability	Membrane integrity
Control		53.02±0.35	54.41±1.10	56.08±1.31
Glycine	5 mM	58.10±0.77^{a}*	57.91±0.49*	58.45±0.50*
	15 mM	57.38±0.26^{ab}*	57.41±0.86*	58.25±0.52*
	25 mM	55.95±0.80^{b}*	57.58±0.21*	58.50±0.54*
Glutamine	5 mM	57.86±0.80^{a}*	57.91±0.66*	59.66±0.6a*
	15 mM	60.39±0.77^{b}*	58.66±0.51*	59.58±0.58*
	25 mM	59.11±0.42^{ab}*	58.08±0.58*	59.50±0.71*
Cysteine	3 mM	55.10±0.93^{a}*	55.16±1.47^{a}	56.83±1.32^{a}
	5 mM	57.16±1.16^{b}*	57.50±0.54^{b}*	59.11±0.77^{b}*
	7 mM	55.83±1.47^{ab}*	55.66±1.03^{a}	57.00±1.54^{a}

Within each amino acid, means with different alphabetical superscripts are significantly different (P<0.05)
Within columns, (*) indicates significant difference (p<0.05) of a given element from control

Table 2. Effect of different concentrations of selected amino acids on characteristics of SO bull spermatozoa post thawing (Mean ± SE)

Treatment		Semen characteristics		
		Motility	Viability	Membrane integrity
Control		47.38±0.44	51.02±0.63	52.67±0.79
Glycine	5 mM	49.51±0.61^{a}	52.85±0.35	54.02±1.02
	15 mM	52.53±1.68^{b}*	54.50±1.37*	54.22±0.92
	25 mM	50.35±1.18^{ab}*	53.75±1.66*	52.97±1.81
Glutamine	5 mM	49.60±2.18^{a}	53.58±0.80*	54.25±1.89
	15 mM	52.98±1.43^{b}*	53.58±1.15*	54.75±1.03
	25 mM	50.87±1.22^{ab}*	53.16±1.63	53.75±2.42
Cysteine	3 mM	47.50±1.22^{a}	52.83±2.04	51.50±2.58
	5 mM	51.00±2.19^{b}*	55.00±1.26*	54.33±1.63
	7 mM	48.66±0.81^{ab}	52.83±0.75	53.00±1.67

Within each amino acid, means with different alphabetical superscripts are significantly different (P<0.05)
Within columns, (*) indicates significant difference (P<0.05) of a given element from control

on the sperm membrane which resulted in irreversible damage to intracellular structures and changes in enzymatic activity and associated reduction in motility, fertilizing ability of spermatozoa (Kumar et al. 2011). Amino acids are the constituents of the seminal plasma and have been used in different combinations in extenders for the cryopreservation of semen in different livestock species (Farshad & Hosseini 2013; Sarıözkan et al. 2014; El-Sheshtawy et al. 2008).

In the present study, the supplementation of amino acids (glutamine, cysteine, glycine) to TCFY extenders improved the spermatozoa quality with respect to the motility, viability and membrane integrity both after the equilibration and post-thaw. Although, the mechanism of action of the amino acids as a cryoprotectant is still not clear but many authors have reported that the amino acids possess the anti-oxidative capacity (Farshad & Hosseini 2013; Bucak et al. 2009; Topraggaleh et al.

2014) which may be attributed to its cryoprotective nature.

Data resuled showed similarity to the data from previous study (Khalili et al. 2010) that post-thawing sperm motility and viability were significantly improved at concentrations between 5 to 15 mM of glycine and cysteine, and 15 mM glutamine (Purwasih et al. 2013). Kundu et al. (2001) reported that lower concentration (20-70 mM) of glycine and glutamine manifest their cryoprotecting efficacy on goat sperm in the presence of glycerol or dimethyl sulfoxide. Khlifaouia et al. (2005) suggested that glutamine could have synergistic cryoprotective role with glycerol on cryopreservation of stallion spermatozoa and its penetration to sperm cells is very low, thus glutamine may play a cryoprotective role at extracellular level. Ali Al Ahmad et al. (2008) showed that glutamine has a cryoprotective effect during the freezing/thawing process of goat sperm. Previous studies reported that the optimal concentrations of glutamine in cryopreservation extender were 25 mM in goat (Ali Al Ahmad et al. 2008), 10 mM in bull (Amirat et al. 2004), 30 mM (Trimeche et al. 1999) or 50 mM in stallion (Khlifaouia et al. 2005) and in man (Renard et al. 1996).

Result of this research, clearly indicated that the addition of glutamine, glycine, and cysteine in the conventional freezing medium increased post-thawed motility and improved membrane and acrosome integrity of SO bull semen. These results were in line with the results obtained from buffalo bull semen (El-Sheshtawy et al. 2008). It is well known that mammalian spermatozoa contain high concentrations of polyunsaturated fatty acids, and therefore are highly vulnerable to oxidative stress which is responsible for the generation of reactive oxygen species (ROS) (Cassani et al. 2005). Oxidative stress generally leads to loss of motility, swelling and the blebbing of the acrosomal membrane and disruption or increased permeability of the plasma membrane of spermatozoa. Bilodeau et al. (2001) demonstrated that thiol-radicals containing amino acids, such as glutathione, cysteine, N-acetyl-cysteine and 2 mercapto ethanol, prevented hydrogen peroxide mediated loss of sperm motility in frozen-thawed bull semen.

DNA stability of frozen-thawed Sumba Ongole bull spermatozoa

The result observation of the DNA integrity of frozen-thawed spermatozoa are presented in Table 3 and Figure 1.

The DNA integrity of frozen-thawed spermatozoa after fixation with acetic alcohol and stained with acridine orange showed all spermatozoa were green color. These results indicats that all spermatozoa in the control treatment as well as with the addition of amino acids have a stable DNA content. The DNA of mammalian sperm is mostly known as the most compact eukaryotic DNA which is packaged more tightly than the tightly packaged mitotic chromosomes of somatic cells (Ward & Coffey 1991). In this study, identification of DNA damage using acridine orange test, the DNA damaged and intact sperm were classified subjectively under florescence microscope. Acridine orange inserts between nitrogenous bases in double stranded DNA (DS-DNA) as monomers. This makes spermatozoa produced green color when observed with a fluorescence microscope. If the DNA molecule consists of single stranded, acridine orange will join the

Table 3. DNA integrity of frozen-thawed SO cattle spermatozoa using acridine orange method

Treatments		DNA Integrity (%)	
		Intact	No Intact
Control		200 (100.00)	0 (0.00)
Glycine	5 mM	200 (100.00)	0 (0.00)
	15 mM	200 (100.00)	0 (0.00)
	25 mM	200 (100.00)	0 (0.00)
Glutamine	5 mM	200 (100.00)	0 (0.00)
	15 mM	200 (100.00)	0 (0.00)
	25 mM	200 (100.00)	0 (0.00)
Cysteine	3 mM	200 (100.00)	0 (0.00)
	5 mM	200 (100.00)	0 (0.00)
	7 mM	200 (100.00)	0 (0.00)

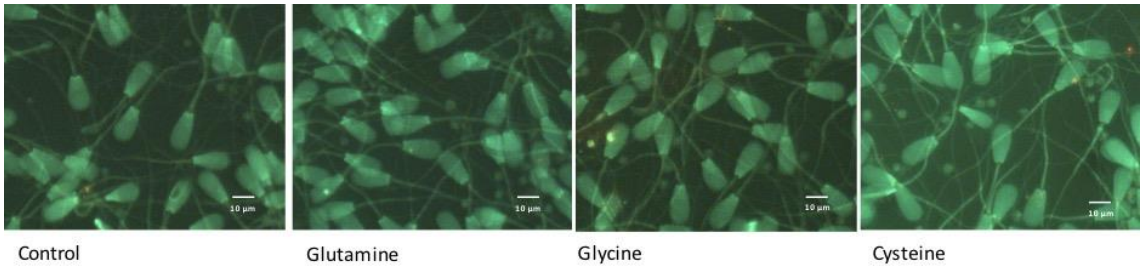


Figure 1. DNA integrity of frozen-thawed SO cattle spermatozoa using fluorescence microscope.

DNA into a single unit, and when observed with a fluorescence microscope, the spermatozoa will produce a red color (Morshedi 2014; Evenson 2016). The color produced by acridine orange in spermatozoa depends on the thiol-disulfide status of the protamine. In immature spermatozoa (immature), the protamine contains thiol. This causes DNA in immature spermatozoa to be denatured become single stranded by acid. When spermatozoa undergo maturation in the epididymis, thiol on the protamine will change gradually into disulfide. This causes DNA in mature spermatozoa to be resistant to acid-induced denaturation, so the DNA will remain double stranded (Said & Niwa 2004).

In the present study, when spermatozoa were stained with acridine orange after fixation with acetic alcohol, the DNA integrity of frozen-thawed spermatozoa showed that all spermatozoa were green color. These results indicate that all spermatozoa in the control treatment as well as with the addition of amino acids have a stable DNA content. Oxidative stress occurs due to the imbalance between the production of reactive oxygen species and antioxidant defense system. It was reported that ROS production pathologically results in high levels of DNA damage that is associated with properties of mitochondrial membrane (Hosen et al. 2015). The structure of the plasma membrane is unique and consists of high levels of polyunsaturated fatty acids (PUFAs) that improve membrane flexibility. It makes them vulnerable to be attacked by ROS (Sheweita et al. 2005). Lipid peroxidation cascade can seriously compromise the functional integrity of membrane cells, decrease sperm motility, and subsequently reduce fertility.

CONCLUSION

In conclusion the current study demonstrated that addition of 15 mM glycine, 15 mM glutamine and 5 mM cysteine increase the cryoprotecting efficacy of SO bull cryopreservation extender, and furthermore all DNA spermatozoa were remain intact. However, further studies still needed to observ the effect of combined amino acids addition.

ACKNOWLEDGEMENT

The authors would like to thank Research Center for Biotechnology, Indonesian Institute of Sciences and PT. Karya Anugerah Rumpin for the permission to use farm facilities. Author gratitude is also expressed to members of Animal Research Group in Research Center for Biotechnology, Indonesian Institute of Sciences.

REFERENCES

Akcay E, Bozkurt Y, Secer S, Tekun N. 2004. Cryopreservation of mirror carp semen. Turkey J Vet Anim Sci. 28:837-843.

Ali Al Ahmad MZ, Chatagnon G, Amirat-Briand L, Moussa M, Tainturier D, Anton M, Fieni F. 2008. Use of glutamine and low density lipoproteins isolated from egg yolk to improve buck semen freezing. Reprod Domest Anim. 43:768.

Amirat L, Tainturier D, Jeanneau L, Thorin C, Gerard O, Courtens J, Anton M. 2004. Bull semen in vitro fertility after cryopreservation using egg yolk ldl: a comparison with optidyl, a commercial egg yolk extender. Theriogenology. 61: 895-907.

Atig F, Raffa M, Ali Habib B, Kerkeni A, Saad A, Ajina M. 2012. Impact of seminal trace element and glutathione levels on semen quality of Tunisian infertile men. BMC Urol. 12:6.

Bilodeau JF, Blanchette S, Gagnon C, Sirard MA. 2001. Thiols prevent H2O2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology. 56:275-286.

Bucak MN, Atessahin A, Abdurrauf Y. 2008. Effect of antioxidants and oxidative stress parameters on ram after the freeze-thawing process. Small Rumin Res. 75:128-134.

Bucak MN, Tuncer PB, Sarıözkan S, Ulutaş PA. 2009. Comparison of the effects of glutamine and an amino acid solution on post-thawed ram sperm parameters, lipid peroxidation and anti-oxidant activities. Small Rumin Res. 81:13-17.

Cassani P, Beconi MT, O'Flaherty C. 2005. Relationship between total superoxide dismutase activity with lipid peroxidation, dynamics and morphological parameters in canine semen. Anim Reprod Sci. 86:163–173.

Chatterjee S, De Lamirande E, Gagnon C. 2001. Cryopreservation alters membrane sulfhydryl status of bull spermatozoa: protection by oxidized glutathione. Mol Reprod Dev. 60:498-06.

El-Sheshtawy RI, El-Sisy GA, El-Nattat WS. 2008. Use of selected amino acids to improve buffalo bull semen cryopreservation. Glob Vet. 2:146-150.

Evenson DP. 2016. The sperm chromatin structure assay (SCSA) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. Anim Reprod Sci. 169: 56-75.

Farshad A, Hosseini Y. 2013. The cryoprotective effects of amino acids supplementation on cooled and post- thaw Markhoz bucks semen quality. Small Rumin Res. 114:258-263.

Hosen MB, Islam MR, Begum F, Kabir Y, Howlader MZH. 2015. Oxidative stress induced sperm DNA damage, a possible reason for male infertility. Iran J Reprod Med. 13:525-532.

Khalili B, Jafaroghli M, Farshad A, Paresh-Khiavi M. 2010. The effects of different concentrations of glycine and cysteine on the freezability of moghani ram spermatozoa. Asian-Aust J Anim Sci. 23:318-325.

Khlifaouia M, Battuta I, Bruyasa JF, Chatagnona G, Trimecheb A, Tainturiera D. 2005. Effects of glutamine on post-thaw motility of stallion spermatozoa: an approach of the mechanism of action at spermatozoa level. Theriogenology. 63:138-149.

Kumar R, Mohanarao GJ, Atreja SK. 2011. Freeze: Thaw induced genotoxicity in buffalo (Bubalus bubalis) spermatozoa in relation to total antioxidant status. Mol Biol Rep. 38:1499-1506.

Kundu CN, Das K, Majumder GC. 2001. Effect of amino acids on cauda epididymal sperm cryopreservation using a chemically defined model system. Cryobiology. 41:21-27.

Kutluyer F, Kocabas M. 2016. Use of amino acids in fish sperm cryopreservation: A Review. Austin Biol. 1:1-4.

Morshedi M. 2014. Human sperm nuclear DNA fragmentation assays and their values in assisted. College of reproductive biology 18th annual symposium: 1-15.

Purwasih R, Ondho YS, Sutopo. 2013. Efektivitas prefreezing semen Sapi Jawa sebagai parameter keberhasilan processing semen beku. Anim Agric J. 2:44-50.

Renard P, Grizard G, Griveau JF, Sion B, Boucher D, Le Lannou D. 1996. Improvement of motility and fertilization potential of post-thaw human sperm using amino acids. Cryobiology. 33:311-319.

Revell SG, Mrode R. 1994. An osmotic resistance test for bovine semen. Anim Reprod Sci. 36:77-86.

Said S, Afiati F, Maulana T. 2015. Study on changes of sperm head morphometry and DNA integrity of freeze-dried bovine spermatozoa. J Indones Trop Anim Agric. 40:145-152

Said S, Funahashi H, Niwa K. 1999. DNA stability and thiol-disulphide status of rat sperm nuclei during epididymal maturation and penetration of oocytes. Zygote 7:249-254.

Said S, Han MS, Niwa K. 2003. Development of rat oocytes following intracytoplasmic injection of sperm heads isolated from testicular and epididymal spermatozoa. Theriogenology. 60:359-369.

Said S. Niwa K. 2004. Keberhasilan ICSI tergantung pada stabilitas DNA dan status disulfida inti spermatozoa. JITV. 9:210-215.

Sarıözkan S, Özdamar S, Türk G, Cantürk F, Yay A. 2014 In vitro effects of l-carnitine and glutamine on motil- ity, acrosomal abnormality, and plasma membrane integrity of rabbit sperm during liquid-storage. Cryobiology. 68:349-353.

Sheweita SA, Tilmisany AM, Al-Sawaf H. 2005. Mechanisms of male infertility: role of antioxidants. Curr Drug Metab. 6:495-501.

Topraggaleh TR, Shahverdi A, Rastegarnia A, Ebrahimi B, Shafiepour V, Sharbatoghli M, Esmaeili V, Janzamin E. 2014. Effect of cysteine and glutamine added to extender on post-thaw sperm functional parame- ters of buffalo bull. Andrologia. 46:777-783.

Trimeche A, Yvon JM, Vidament M, Palmer E, Magistrini M. 1999. Effects of glutamine, proline, histidine and betaine on post-thaw motility of stallion spermatozoa. Theriogenology. 52:181-191.

Tsai S, Lin C. 2012. Advantages and applications of cryopreservation in fisheries Fscience. Braz Arch Biol Technol. 55:425-433.

Ward WS, Coffey DS. 1991. DNA packaging and organization in mammalian spermatozoa: Comparison with somatic cells. Biol Reprod. 44:569-74

White IG. 1993. Lipids and calcium uptake of sperm in relation to cold shock and preservation: a review. Reprod Fertil Dev. 5:639-658.

[WHO] World Health Organization. 2010. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva (Switzerland): World Health Organization. p. 260.

Zar JH. 1974. Biostatistical analysis. London (UK): Prentice-Hall, Inc. p. 620.

The Relationship of Pod Colour with the Quality of *Indigofera zollingeriana* Seed

Hutasoit R, Riyadi, Juniar S

Indonesian Goat Research Station, PO Box 1 Sei Putih, Galang 20585, North Sumatra
E-mail: h.rijanto@yahoo.com

(received 05-10-2018; revised 18-01-2019; accepted 24-01-2019)

ABSTRAK

Hutasoit R, Riyadi, Juniar S. 2019. Hubungan warna polong dengan kualitas benih *Indigofera zollingeriana*. JITV 24(1): 22-28. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1923

Indigofera zollingeriana (Indigofera) berpotensi sebagai bahan pakan. Umumnya perbanyakan tanaman ini melalui benih. Kualitas benih yang rendah merupakan masalah dalam perkembangannya. Penelitian ini bertujuan untuk mengevaluasi hubungan warna polong dengan kualitas benih Indigofera. Penelitian ini dirancang dalam rancangan acak lengkap yang terdiri dari empat warna polong dan empat ulangan, yaitu: P_1 = hijau, P_2 = hijau kecoklatan, P_3 = coklat, dan P_4 = hitam. Parameter yang diamati adalah: karakteristik dan morfologi polong dan biji indigofera, pertumbuhan kecambah, dan pertumbuhan jamur pada benih indigofera. Hasil penelitian menunjukkan bahwa jumlah hama paling sedikit ditemukan di P_2, polong hijau kecoklatan (14%). jumlah benih tertinggi adalah P_1, polong hijau (5173) dan P_2, polong hijau kecoklatan (4944). Daya cambah tertinggi (62%) terdeteksi di P_2 (hijau kecoklatan). Tunas terberat berada di P_2 pada polong hijau kecoklatan (0,035g), tunas tertinggi (2,68 cm) terdapat pada P_4 dengan polong berwarna hitam. Berdasarkan pengamatan jamur, polong hitam (P_4) memberikan hasil paling sedikit (6,63%), namun sebagian besar jamur tumbuh sangat baik di P_1 polong hijau (47,88%). Dapat disimpulkan bahwa warna polong hijau kecoklatan adalah fase terbaik untuk panen benih I. zolligeriana berkualitas baik.

Kata Kunci: Benih *Indigofera zollingeriana*, Warna Polong, Germinasi, Tunas

ABSTRACT

Hutasoit R, Riyadi, Juniar S. 2019. The relationship of pod colour with the quality of *Indigofera zollingeriana*. JITV 24(1): 22-28. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1923

Indigofera zollingeriana (Indigofera) plant is potential feed ingredients. The propagation of this plant is through seed. The low quality of seed is a problem in its development. This study was aimed to evaluate the relationship of pod colour with quality of Indigofera seeds. The study was designed in a complete randomized design consisting of four pod colours and four replications, namely: P_1 = green, P_2 = brownish green, P_3 = brown, and P_4 = black. The parameters observed were: characteristic and morphology of pods and seeds of Indigofera, the growth of sprouts, and the growth of fungus on Indigofera seed. Results showed that the number of pests was fewest found in P_2, brownish green pod (14%). The highest number of seeds was in P_1, green pod (5173) and P_2, brownish green pod (4944). The highest germination (62%) was detected in P_2 (brownish green). The heaviest sprout was in P_2, in brownish green pod (0.035g), highest sprout (2.68 cm) in P_4, black pod colour. Based on fungus observation, the black pod (P_4) provided the fewest result (6.63%), however most fungus grew very well in P_1, the green pod (47.88%). It could be concluded that the brownish green pod colour was the best phase for harvesting good quality *I. zolligeriana* seed.

Key Words: *Indigofera zollingeriana* Seed, Pods Colour, Germination, Sprout

INTRODUCTION

One of the most popular forages is *Indigofera zollingeriana* (Indigofera). It has very high nutrient content, is potential as feed ingredient, and is able to increase animal productivity (Salvador et al. 2010; Suharlina et al. 2016; Telleng et al. 2017). In Indonesia, the development of Indigofera has been quite good and has been spread almost throughout the provinces with various agro-ecosystems. In addition, Indigofera is also very easy to plant with seeds. The seeds are usually obtained from black pods, harvested in the field through drying and milling process to separate the seeds from the pods.

The demand of Indigofera seed is increasing every year. On the other hand, the providers can not fulfill all the demands. Low production and quality of seeds, contaminating pest and fungus on the sprouts were most likely due to the lack of harvesting management. The available information obtained by increasing Indigofera seed productivity remains limited. The previous research reported by Kissock & Haferkampa (1983), presowing seed treatment and temperature affect germination of *Indigofera miniata* var *leptosepala*

obtained an average sprout growth of 63%. Abdullah (2014) reported percentage of indigofera seed germination was 28-35% and increased 67-74% by giving organic matter. Girsang (2012) reported that CO_2 injected into the seed of Indigofera resulted in 36% germination at the 10% level of CO_2. Comparing 5 different soaking temperatures, Hutasoit et al. (2017) obtained the highest sprout growth (50%) at the 100°C. These results could not increase the productivity of Indigofera seeds.

There were no reports on harvesting management of Indigofera seed, especially the time and characteristics of good pods to be harvested. According to our observations at the time of harvesting, there are some different colour of Indigofera pod; namely: green, brownish green, brown, and black. In the green pod, Indigofera seeds look clean from the pests and most likely good to harvest, but the seeds are still in soft and wet condition. In the brownish green pods, the seed conditions are slightly hardened, but it already shows the spot of pest attacks. In the brown pod, the seed conditions are old and hardened, but they have attacked by some pest, marked by eggs form and rotten liquids. In black colour pods, the seeds are old and pithy. Generally, the seeds were harvested at this condition. Nevertheless, many seeds in this phase were broken, hollow, have no seed meat, and have borer pest. So far, there is no information about the kind of this pest, and unknown pest species attacking the seeds. In *Leucaena leucochepala*, the pest, *Acanthoscelides macrophthalmus* caused damage of the seed (Effowe et al. 2010), resulted in 50% of seeds could not be used. While in food crops, *Etiella* spp pod borer can cause damage on soybean and high damage since the larval life is very long until the age of 18 days (Ernestina 2003).

Besides the ageing plant, the pod colour is also very decisive in the harvesting of seeds in order to minimize the attacks of pests and fungi. Previous studies reported several good pod colour phases for harvesting such as yellowish brown colour on *Gliricidia* (Winata et al. 2012), pale brown (russet) on *Calopogonium* (Rahmawathi 2017), orange brown on *Leucaena leucocephala* (Al-Bugg 2017), reddish brown on bambara peanut (Hindun 2013), and blackish brown on soybean (Harnowo 2015).

There are only few information on pod colour of Indigofera seed to harvest. Therefore, the purpose of this research was to evaluate the relationship of pod colour with the quality of *Indigofera zollingeriana* seed.

MATERIAL AND METHODS

This activity was carried out in experimental field and laboratory of Indonesian Goat Research Station, Deli Serdang Regency of North Sumatera, located at an altitude of ± 50 m above sea level with an average temperature of 27°C, 70% humidity and rainfall of 1800 mm/year. The study was conducted from February to May 2018. The seeds of feed plant used are *Indigofera zollingeriana*, as many as 400 twigs of pods. This study was conducted using a Completely Randomized Design (CRD) consisting of four pod colours. Namely: P_1 = green, P_2 = brownish green, P_3 = brown, and P_4 = black. Each treatment has four replications so that there are 16 treatments in this study. The seeds of Indigofera were harvested from the experimental field of Indonesian Goat Research Station. The pods were separated based on the colour; ie: green, brownish green, brown, and black pod. Each colour of pod was taken as many as 100 pod twigs for observation. The variables measured were as follow:

Characteristics and morphology of pods and seeds of Indigofera

Characteristics observed in this study were:

1. Number of pods. One hundred pods were obtained from separation of pods and twigs
2. An average number of pods/twigs
3. Fresh weight of pods by weighing the pods obtained from each group of pod colour
4. The dry weight of pods. The obtained pods were dried in the sun for three days and weighed
5. Percentage of pod dry weight

(A): Green pod; (B): brownish green pod; (C): Brown pod; (D): Black pod

Figure 1. Indigofera pod colour performance**.**

6. Length of the pod. There were as many as 50 fresh pods, were measured between one tip to the other tip of pod with ruler
7. The diameter of the pod was measured by means of round pods using the yarn then the yarn was measured using ruler
8. A number of pods attacked by pests. A total of 50 fresh pods were observed for its pest attacks marked by the presence of holes in pods
9. A total number of seeds. The dried pods were milled using a blender, then sieved and cleaned and then calculated the number of seeds obtained
10. Seed weight. The seeds obtained were weighed
11. A number of seeds/pods. A number of seeds in one gram was then counted
12. The shape of round and flat seed. As many as one gram of seeds were separated based on the shapes of the round and flat

The sprout growth of Indigofera seeds

The Indigofera seed sprout test was performed in the laboratory using materials such as petri dish, measuring cup, gas stove (heater), thermometer, water and cotton. The seeds obtained from each group of color were sprout growth test. The seeds of Indigofera were soaked in a glass with a temperature of 100°C and left for one night (12 hours), and then the water was drained. The seeds were sowed on wet cotton laid in a bottom of a petri dish. Each group of pod color consisted of 100 tested seeds multiplied by four replications. Sprout growth was observed from 3 to 20 days after seeding.

The parameters observed were:

a. The growth of sprout (Gos)
   The growth of sprout was measured by counting the number of sprouts growing on the 20th day, which was estimated no more sprouts were growing, then calculated the percentage of sprouts growing with the formula:

$$Gos = \frac{\Sigma \text{ sprouts} \times 100\%}{\Sigma \text{ Total seeds planted}}$$

b. The weight of sprouts
   The weight of the sprouts was obtained by collecting all the growing sprouts, then weighed using the electric scales capacity of 125 g. Furthermore, the weight obtained divided by the number of sprouts.

c. The measurement of sprouts
   The measured Indigofera sprouts by the root primer length and the height of the sprouts using a measuring instrument (ruler). Primary root length measurements start from the beginning to the tip root, while the height of the sprouts is measured from the base to the tip of the sprouts.

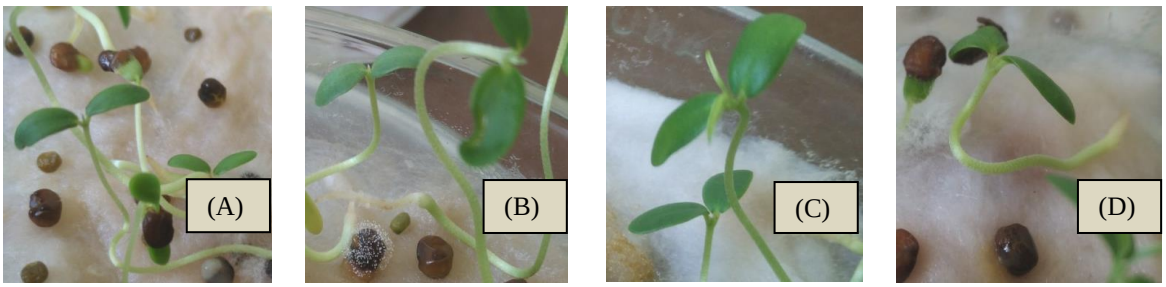
d. The number of sprout leaves
   To obtain the number of sprout leaves (Nos) was done by the formula:

$$Nos = \frac{\Sigma \text{ sprout of leaves}}{\Sigma \text{ growing sprouts}}$$

The growth of fungus (Gof)

The growth of fungus in Indigofera sprout could be seen with nacked-eyes (macroscopic). Symptoms that appear were a fungus characterized by the existence of white hyphae threads and black spots (Meilan et al. 2014). The appearance of fungal colonies at the time of seed germination was calculated by the following formula:

$$Gof = \frac{\Sigma \text{ the growth of fungus} \times 100\%}{\Sigma \text{ planted seeds}}$$



(A): Sprouts from the green pods; (B): Sprouts from the brownish green podst; (C): Sprouts from the brown pods; (D): Sprouts from the black pods.

Figure 2. Sprouting performance of Indigofera seeds selected from four groups of pod color.

Statistical analysis

The data obtained were analyzed with a linear model using SAS software (2009). Significant differences among treatment means were tested using Duncan's multiple range test (DMRT) at the 5% level of.

RESULTS AND DISCUSSION

Characteristics and morphology of pods and seeds of Indigofera

The morphological and characteristics of several phases of the pods and seeds of Indigofera are presented in Table 1. One hundred twigs of samples in each pod colour used in this observation, the number of pods obtained from green, brownish green, brown and black were 1784, 1676, 1394, and 997 pods, respectively. The highest number of seed was found in green pods, and the lowest was detected in the black pod. The data of fresh weight and dry weight of pod seeds showed that although the fresh weight was higher (18.70 g) in the green pod, however the dry weight of seeds obtained was very low (26.20%) indicating that the seeds contained high moisture inside the pods. Although, the black pod colour has the lowest weight (300 g), but the percentage of dry weight obtained was quite high (86.67%). The average diameter pod (4.32 mm), almost the same for all treatments. The longest pod was green pods (36.43mm) and the shortest was in brown colour (33.5mm). The largest pod diameter (4.6 mm) was in brownish green colour, while the smallest were 4.15mm found in black colour pods. The number of pods affected by pests was mostly found in black pod (49%) and the lowest was detected in brownish green (14%) indicating that the black pod colour has been attacked by some pests.

This was most likely because the time for green pod to become black pod took about two months, which was plenty of time for pests to grow and damage the pods. While the green pod formation only took about a month which was less time to be damaged by the pest.

Observations on seed production, green pod colour had the highest number of seeds (5,173), weighing 20.3g, while the brownish green pods had 4,944 seeds, with total weigh of seeds was 24.24g. The lowest number of seeds were in brown and black pods of 3,792, and 3,589 seeds, weighing 23.09 and 18.99g, respectively. The number of seeds/pods of each colour was moderate and nearly same (average 3.07); the highest (3.6) in the black pods and the lowest (2.9) in the green. The number of seeds/g was mostly in green pods (252) and brownish green (204), less in brown (172) and black (189). The shape of round seeds was the highest (73%) in brownish green while the flat shape was the highest (53%) in green pod.

Table 1. The characteristics morphology of pod and seed of Indigofera

Parameters	The colour of pods				Average
	Green	Brownish green	Brown	Black	
Number of pod	1784	1676	1394	997	1462.75
Average of pod/twig	17.84	16.76	13.94	9.97	14.63
Fresh of pod weight (g)	1870	590	470	300	807
Dry weight (g)	490	300	340	260	347.50
Dry weight (%)	26.20	50.85	72.34	86.67	59.01
Length of pod (mm)	36.43	34.92	33,5	34.33	3479
Diameter of pod (mm)	4.3	4.6	4.25	4.15	4.32
Pods attacked by pests (%)	22	14	29	49	28.5
The total number of seeds	5173	4944	3972	3589	4419
The weight of seeds (g)	20.53	24.24	23.09	18.99	21.71
Number of seeds/g	252	204	172	189	204.25
Average of seeds/pod	2.9	2.95	2.85	3.6	3.07
Round seeds (%)	47	73	67	63	62.5
Flat seeds (%)	53	27	33	37	37.5

The sprout growth of Indigofera seeds

The results of the study to the sprout growth of Indigofera seed are shown in Table 2. The average percentage of the sprout growth was 57.75%. The highest percentage (P<0.05) was found in brownish green pod (62%). Sprout growth was lower than the normal standard. According to the Directorate General of Food Crops (1991), the standard (SNI = Indonesian National Standard) of seed that was able to sprout should be minimum 75% and the plant should be able to grow normal under suboptimum conditions. The weight of sprout was significantly (P<0.05) the heaviest (0.035 g) from the seeds in brownish green pods, compared to those in the other pod colours, indicating that the plant would grow strong.

The average heights of sprouts are 2.68cm, a range of 2.35 cm (green) – 3.18 cm (black). The height of sprouts from black pod was significantly different (P<0.05) from three other colours, indicating that they would have the higher growth and production (Risva et al. 2014).

Primary root length showed that the seeds from brownish green, brown, and black were relatively same and did not differ significantly, achieving of length of 1.67, 1.74, and 1.68 cm respectively; they were significantly longer (P<0.05) than that of seeds obtained from the green colour (1.42cm). The length of the root of all sprouts of seeds from previous three colors could spur the growth of plants and increase the ability to absorb water and dissolved nutrients (Hidayanto et al. 2003; Nio & Patricia 2013).

The average number of sprout leaves of seeds obtained from brownish green, brown, and black pods did not differ significantly (P<0.05). While the average number of leaves counted from seeds derived from green pods was the lowest (1.61 cm). This was most likely due to the low content of gibberellin in young seeds indicating that the growth of the content of the seed substance was still low. Weiss & Ori (2007) reported that one of the physiological effects of gibberellin was to encourage the activity of hydrolytic enzymes in seed germination processes.

The growth of sprouts according to the shape of Indigofera seeds is shown in Table 3. The results obtained indicate that the shape of the seed does not affect seed germination. However, it has a significant effect on the height of sprout, length primary root, and number of leaves. Round seed growth is superior compared to the flat, has the highest germination (3.12 cm), the primary root length was 2.02 cm, and the number of leaves of 2.14 strands. There was significantly different (P<0.05) to the growth of flat seeds (2.15 cm, 1.23 cm and 1, 6 strands) respectively. The high levels of growth of sprout in the round seeds is agreement with previous reports (Gusta et al. 2003), on Maize seedlings showed that the types of round seed was higher in growth rate than the flat one. This is most likely due to the cotyledon of the round seeds was wider, so that the photosynthetic potential is higher than the flat seeds.

Table 2. The growth of sprouts of Indigofera seeds from four different colour of pod

Parameter	The colour of pods				Average
	Green	Brownish green	Brown	Black	
Growth of sprouts (%)	55^{b}	62^{a}	57^{b}	45^{c}	57.75
Weight of Sprouts (g)	0.024^{d}	0.035^{a}	0.032^{b}	0.030^{c}	0.030
Height of sprout (cm)	2.37^{c}	2.35^{c}	2.81^{b}	3.18^{a}	2.68
Length of primary root (cm)	1.42^{b}	1.67^{a}	1.74^{a}	1.68^{a}	1.63
Number of leaves (strands, cm)	1.61^{c}	1.81^{a}	1.82^{a}	1.75^{a}	1.75

Values with different superscripts are significantly different (P<0.05)

Table 3. The growth of sprouts of Indigofera seeds based on different shape

Parameter	The shape of seed	
	Round seeds	Flat seeds
Growth of sprout (%)	59.76^{a}	54.84^{a}
Height of sprout (cm)	3.12^{a}	2.15^{b}
Length of primary root (cm)	2.02^{a}	1.23^{b}
Number of leaves (strands)	2.14^{a}	1.6^{b}

Values with different superscripts are significantly different (P<0.05)

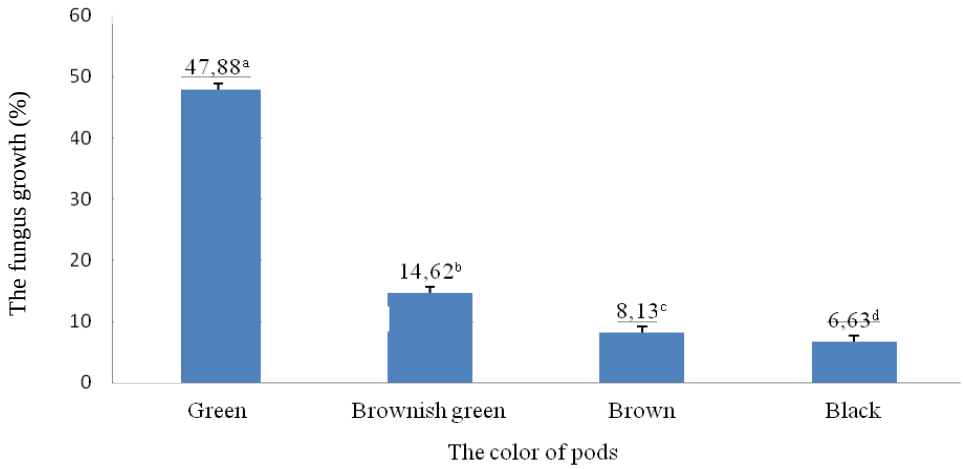


Figure 3. The fungus growth from different pod colour.

In Figure 3, it is shown that the darker of pod colour has the lower growth of the fungus. The lowest number of fungi was from black pod colour seeds which was only 6.63% ($P<0.05$) compared to the other three pod colours (8.13%, 14.62%, and 47.88%, respectively). This was most likely related to the water content of each colour phase. In tune with the dry matter content of the seeds in Table 1 the highest water content was found in green pod, and slightly present in black. The higher the moisture content is, the more fungi growth. It was suggested by Suryanto (2013), that water content in seed should be controlled to be as low as 10.82% to get germination up to 71%.

The fungus growth

Fungi are the main pathogenic causes of seed damage. Some fungi in seeds such as *Alternaria sp, Fusarium oxysporum, F. solani, F. equiseti, Myrothecium roridum, Drechslera sp,* are the most dangerous pathogen causing pathogenic disease since they depressed the health of plant (Bakr & Rahman 2001). The low water content in seeds could inhibit fungal growth, and high water content caused high humidity. This condition could stimulate the growth of fungus during storage (Sudir et al. 2014). The moisture may induce fungi- airborne pathogens which contaminate the Indigofera seeds as well as the emergence of fungal colonies at the time of seed germination

CONCLUSION

The brownish green pod color of *Indigofera zollingeriana* seeds is well used against sprout growth, sprout weight, primary root length. While the black color of the pods has the least fungal growth.

REFERENCES

Al-Bugg YSH. 2017. Seeds, pods and petiole gland's characteristics of Leucaena spp. analytical study (a). Int J Res-Granthaalayah. 5:421-434.

Abdullah L. 2014. Prospektif agronomi dan ekofisiologi Indigofera zollingeriana sebagai tanaman penghasil hijauan pakan berkualitas tinggi. Pastura. 3:79-83.

Bakr MA, Rahman ML. 1998. Current status of research on mungbean and black gram diseases and future needs. Proceeding of the workshop on diseases resistance breeding in pulse. Bangladesh J Agric. 11:64-72.

Directorate General of Food Crops. 1991. Petunjuk Pengawas Benih. Jakarta (Indones): Direktorat Jendral Pertanian Tanaman Pangan – Direktorat Bina Produksi Padi dan Palawija Sub Direktorat pengawasan Mutu dan Sertifikasi Benih.

Effowe, TQ, Amevoin K, Nuto Y, Mondedji D, Glitho IA. 2010. Reproductive capacities and develop ment of a seed bruchid beetle, *Acanthocelides macrophthalmus*, a potential host for the mass rearing of the parasitoid, *Dinarmus basalis*. J Insect Sci. 10:129-136.

Ernestina F. 2003. Pengaruh fase perkembangan polong dan trikoma terhadap preferensi penularan hama penggerek polong kedelai *Etiella zinkenella* treit. [Skripsi]. [Malang (Indonesia)]: Fakultas Pertanian, Universitas Brawijaya.

Girsang RC. 2012. Viabilitas Benih Indigofera (*Indigofera zollingeriana*) setelah Injeksi Karbon Dioksida (CO2) dan Penyimpanan [Skripsi]. [Bogor (Indonesia)]: Institut Pertanian Bogor.

Gomes J, Bayer C, De Souza Costa F, De Cássia Piccolo M, Zanatta JA, Vieira FCB, Six J. 2009. Soil nitrous oxide emissions in long-term cover crops-based rotations under subtropical climate. Soil Tillage Res. 106:36-44.

Gusta LV, Johnson EN, Nesbit NT, Kirkland KJ. 2003. Effect of seeding date on Canola seed vigor. Can J Plant Sci. 45:32-39.

Harnowo D. 2015. Prinsip-prinsip produksi benih kedelai. In: Didik H, Marwoto, Muchlish A, Titik S, Novita N., editors. Malang (Indones): Balai Penelitian Tanaman Aneka Kacang dan Umbi. p. 10-13.

Hindun, Wicaksana N, Waluyo B, Rachmadi M, Karuniawan A. 2013. Karakteristik fisik polong dan biji kacang bambara (*Vigna subterranea* (L). Verdc.). Budi W, Agung K, Noladhi W, editors. Prosiding Seminar Nasional 3 in one Malang. Malang (Indones): Fakultas Pertanian, Universitas Brawijaya. p. 430-435.

Hidayanto M, Siti N, Yossita F. 2003. Pengaruh panjang stek akar dan konsentrasi natrium nitrofenol terhadap pertumbuhan stek akar sukun. J Pengkajian dan Pengembangan Teknologi Pertanian. 6:154-160.

Hutasoit R, Riyadi, Ginting SP. 2017. Pengaruh suhu perendaman terhadap pertumbuhan kecambah benih *Indigofera zollingeriana*. Puastuti W, Muharsini S, Inounu I, Tiesnamurti B, Kusumaningtyas E, Wina E, Herawati T, Hartati, Hutasoit R, et al., editors. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor (Indones): Pusat Penelitian dan Pengembangan Peternakan. p. 531-538.

Kissock DC, Haferkampa MR. 1983. Presowing seed treatment and temperature effects on germination of *Engezmannia pinnatifida* and *Indigofera miniata* var.leptosepala. J Range Manag. 36:94-97.

Meilan S, Azis P, Sulandari S. 2014. Pengaruh pemanasan terhadap perkecambahan dan kesehatan benih kedelai (*Glycine max* (L.) Merrill). J Vegetalika. 3:27-37.

Nio SA, Patricia T. 2013. Karakter morfologi akar sebagai indikator kekurangan air pada tanaman. J Bioslogos. 3:32-39.

Rahmawathi AM. 2017. Sistem agroforestri sentang (*Azadirachta excelsa* (jack) m. jacobs) dengan kedelai (*Glycine max* (l.) merr.) secara organik. [Thesis]. [Bogor (Indones)]: Institut Pertanian Bogor.

Risva AH, Tohari, Sri NHU. 2014. Takaran pupuk nitrogen dan silika terhadap pertumbuhan awal (*Saccharum officinarum* L.) pada inceptisol. Vegetalika. 3:35-44.

SAS Institute Inc. 2009. SAS/STAT 9.2 User's Guide. Cary, NC (USA): SAS Inst Inc.

Salvador I de S, Medeiros RMT, Pessoa CRM, Dantas AFM, Júnior GS, Riet-Correa F. 2010. Intoxicação por *Indigofera suffruticosa* (Leg. Papilionoideae) em bovinos. Pesqui Vet Bras.

Sudir, Nasution A, Santoso, Nuryanto B. 2014. Penyakit Blas Pyricularia grisea pada tanaman padi dan strategi pengendaliannya. Iptek Tanaman Pangan. 9:85-96.

Suharlina, Dewi A, Nahrowi, Jayanegara A, Abdullah L. 2016. Nutritional evaluation of dairy goat rations containing *Indigofera zollingeriana* by using *in vitro* Rumen Fermentation Technique (RUSITEC). Int J Dairy Sci. 11:100-105.

Suryanto H. 2013. Pengaruh beberapa perlakuan penyimpanan terhadap perkecambahan benih suren (*Toona sureni*) (effects of storage of suren (*Toona sureni*) seeds on germination). J Pen Kehut Wallacea. 2:26-40.

Telleng M, Wiryawan KG, Karti PDMH, Permana IG, Abdullah L. 2017. Silage quality of rations based on in situ sorghum-indigofera. Pak J Nutr. 16:168-174.

Weiss D, Ori N. 2007. Mechanisms of cross talk between gibberellin and other hormones. Plant Physiol. 144:1240-1246.

Winata, Nash, Karno, Sutarno. 2012. Pertumbuhan dan produksi hijauan gamal (*Gliricidia sepium*) dengan berbagai dosis pupuk organik cair. Anim Agric J. 1:797-807.

Generation of scFv-Monoclonal Antibody Avian Influenza Diagnostic Tests

Tarigan S, Sumarningsih

Indonesian Research Center for Veterinary Sciences
RE. Martadinata St. No. 30 Bogor

(received 28-09-2018; revised 25-01-2019; accepted 28-01-2019)

ABSTRAK

Tarigan S, Sumarningsih. 2019. Pembuatan antibodi monoklonal-scFV untuk Uji Diagnostik Avian Influenza. JITV 24(1): 29-38. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1871

Kebutuhan alat diagnostik cepat atau *point-of-care diagnostic test* untuk penyakit Avian Influenza di Indonesia sangat besar. Sampai saat ini alat diagnostik tersebut diimpor sehingga harganya mahal. Akibatnya, pengadaannya membutuhkan anggaran yang besar. Komponen utama alat diagnostik cepat adalah antibodi monoklonal yang spesifik terhadap virus influenza. Penelitian ini bertujuan memproduksi mAb yang bisa mengenali semua subtipe Avian Influenza menggunakan teknologi *phage display*. Influenza-A *focused* scFv *library* komersial di *panning* menggunakan rekombinan NP H1N1 dan virion H5N1 secara bergantian. Sedangkan bakteriofag yang terikat pada antigen *panning* dielusi dengan serum dari ayam yang memiliki antibodi terhadap virus H5N1. Phagemid dari *suppressor E. coli* (TG1) yang terinfeksi bakteriofag yang menampilkan anti-NP pada permukaanya diisolasi lalu ditransformasikan pada *non-supressor E. coli* (HB2151) untuk mengekspresikan NP-scFv. Antibodi monoklonal NP-scFv dengan berat molekul sekitar 27 kDa dipurifikasi dari supernatan biakan menggunakan kolom kromatografi nikel. Jumlah NP-scFv murni yang diperoleh adalah sekitar 1.2 mg/L biakan. Sebagai komponen tambahan untuk penggunaanya dalam *immunoassay*, antibodi terhadap NP-scFv diproduksi pada kelinci. Antibodi poliklonal yang dihasilkan mengenali NP-scFv dengan spesifik dan sensitif. Antibodi monoklonal anti-NP-scFv dan poliklonal anti scFv yang dihasilkan dalam penelitian ini dapat digunakan untuk pengembangan alat diagnostik *point -of- care* Avian Influenza.

Kata Kunci: Avian Influenza, Nukleoprotein, Antibodi scFv, Alternating Panning, Uji POC

ABSTRACT

Tarigan S, Sumarningsih. 2019. Generation of scFv-monoclonal antibody Avian Influenza Diagnostic Tests. JITV 24(1): 29-38. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1871

The need for rapid diagnostic tools or point- of- care diagnostic tests for Avian Influenza in Indonesia is very high and the price of these imported diagnostic tools is very expensive. As a result, a large budget requires to provide the needs. The main component of a rapid diagnostic tool is the monoclonal antibody (mAb) specifically recognized influenza viruses. The objective of this study was to produce mAb that can recognize all subtypes of Avian Influenza viruses using the phage display technology. Influenza-A focused scFv commercial library was panned using alternating recombinant H1N1 NP and H5N1 virions. Whereas, bacteriophages bound to the panning baits were eluted with serum from H5N1-infected chickens. Phagemid from suppressor *E. coli* (TG1) infected with bacteriophage displaying anti-NP on its surface was isolated and then transformed into a non-suppressor *E. coli* (HB2151) to express NP-scFv. Monoclonal NP-scFv antibody with a molecular weight of about 27 kDa was purified from the culture supernatant using a nickel-chromatography column. The amount of pure NP-scFv obtained was around 1.2 mg /L culture. As an additional component for its use in immunoassays, antibody to NP-scFv was produced in rabbits. The generating polyclonal antibody recognized the NP-scFv specifically and sensitively. The anti-NP-scFv monoclonal antibody and the anti rabbit scFv polyclonal antibody produced in this study are envisaged appropriate for the development of diagnostic tools for point-of-care for Avian Influenza.

Key words: Avian Influenza, Nucleoprotein, scFv Antibody, Alternating Panning, POC Test

INTRODUCTION

The H5N1 avian influenza seems to be one of the most devastating zoonotic diseases ever known to date (FAO 2013). One of the main factors causing the rapid, wide spread of the disease was the delay in diagnosis and implementing actions to eradicate the disease. Diagnosis of infectious diseases that spread rapidly such as AI H5N1 requires the availability of rapid or point-of -care diagnostic (POC) tools. Most of the POC diagnostic tools for Influenza both for human and poultry are based on monoclonal antibody specific against the nucleoprotein of the type A Influenza virus (Tarigan 2016). The main advantages of using monoclonal include batch-to-batch homogeneity and excellent specificity. Polyclonal antibodies are much easier, cheaper and faster to produce but the variability between different batches produced in different animals at different times is unavoidable. In addition, since polyclonal antibody comprises huge number of

antibodies recognizing different epitopes, cross-reaction is inescapable (Liu 2014; Shalit et al. 1985).

Two approaches to produce monoclonal antibodies are currently available. The first approach was the hybridoma technology introduced by Kohler and Milstein (Kohler & Milstein 1975). This approach involves fusion of B-cells from immunized donor animal with myeloma cell to generate immortal cells producing monoclonal antibody. Some drawbacks of this approach include the use of animals and the limited species of animal as the source of antibody that can be used. So far, myeloma cells that available for that purpose are only mouse and rat origin (Liu 2014).

The second approach for production of monoclonal antibody is the phage display technology introduced in the late 20th century (Smith & Petrenko 1997). Briefly, the approach began by isolation of mRNA from B-lymphocites from donor animals or human, either naïve or immune to a relevant antigen. The heavy variable (VH) and light variable (VL) segments are amplified and connected with a short linker with PCR then batch-cloned into a special phagemid vector, next to the pIII protein of filamentous bacteriophage. A competent *E. coli* strain is transformed with the phagemid and is rescued with a helper phage to derive a single chain variable fragment (scFv)-phage library. Each phage in the library recognizes different epitope through the scFv that fused to the bacteriophage surface protein PIII. The diversity of a scFv library is usually in the range of millions to trillions. The next most important step is to select and purify scFv-phages that recognize the desired antigen by the protocol known as panning. Phagemids from the *E. coli* harboring scFv phage are isolated and transformed into competent cells of a non-suppressor strain of *E. coli* in order to express the scFv antibody (Clackson et al. 1991; Hoogenboom et al. 1998).

Production of mAb using the phage display approach offers many benefits. Once a library is made or purchase commercially, the same library can be used to generate many different mAbs. The production does not require the use of animal. Unlike maintaining hybridoma, which requires liquid nitrogen; maintaining and storage of *E. coli*, phagemid and bacteriophage for future mAb production are easy. The molecule of phage-display mAb is ease to modify; such tagging with other peptides or increase its affinity through an affinity maturation process. The phage-display mAbs penetrate tissue more easily because of its small molecular size (Liu 2014; Nissim et al. 1994; Thompson et al. 1996).

The aim of the present study was to produce mAb recognizing a common antigen for type-A- influenza viruses, the nucleoprotein, using the phage display technology. The mAb is envisaged suitable as the main component of POC test for influenza in animals and human.

MATERIALS AND METHODS

Library and *E. coli* strain

Ready-to-panning H1N1 library (human scFv) was obtained from Oak Bioscience, Sunnyvale, CA (USA). This H1N1-focused library, which was developed from human B-cells, had a diversity of 10^5 and a titer of 10^{11} plaque forming unit (pfu)/ml. Helper phage ($M_{13}O_7$) and *E. coli* TG1 (K-12 glnV44 thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5($r_K^- m_K^-$) F′ [traD36 proAB$^+$ lacI^q lacZΔM15]) and HB2151 *E. coli* (K12 (lac-pro), ara, nalr, thi/F'[proAB, lacIq, lacZ M15] from Creative Biolab, Shirley, NY, USA. Recombinant nucleoprotein H1N1 Influenza virus (NP), anti-M13 monoclonal antibody (Sino Biological, China), and anti-H5N1 serum was obtained from chicken that had been vaccinated and infected with life H5N1 virus in our previous study (Tarigan et al. 2015). The H5N1 sub-type influenza virus was an Indonesian isolate, previously isolated from chicken (Tarigan et al. 2015).

Delipidation of H5N1 virion

The purpose of removing lipid from the H5N1 virion is to increase the binding capacity of viral proteins to polystyrene immunotube or plate. Delipidation was carried out according to a previous method (Cham & Knowles 1976). Briefly, virus suspension in PBS (10^7 EID_{50}/ml) was mixed with 2 volumes of butanol and *di*-iso prophyl ether mixture (40% : 60%). After shaking for 60 min, the mixture was centrifuged (1000 x *G*, 10 min) and the organic phase was discarded. An equal amount of ethyl ether was added to the aqueous phase, shaking and centrifuged as previously. This ethyl-ether treatment was repeated in order to remove residual butanol. Finally, the delipidated virion suspension was aliquoted and freeze-dried.

Panning

Two immune tubes were coated at 4ºC overnight with NP (2 µg in 1 ml) and delipid H5N1 virus (10^7 in 1 ml carbonate buffer, pH 9.6), respectively. After washing 4 times with PBST (PBS plus 0.05% Tween-20) and blocking with 1% bovine serum albumin (BSA) for 2 hr, 1 ml H1N1 library containing 10^{11} pfu in 1 ml PBS was added and incubated for 2 hr at 37ºC. After removing unbound phages by washing 10 times with sterile PBST and twice with PBS, 1 ml chicken-anti-H5N1 serum (diluted 1: 50 in 2YT broth) was added, incubated at 37ºC for 30 min with 250 rpm shaking to release phages bound to NP or H5N1.

The eluted phage suspension was filtered (0.2 µm pore) and added to 9 ml log-phase TG1-*E. coli*. After incubation stationarily at 37ºC for 30 min, 20 ml 2YT broth containing 2% glucose and 150 µg/ml carbencillin were added and incubated at 37ºC, 250 rpm shaking until mid-log phase (A_{600} = 0.5). Helper phage 2.4 x 10^{11} pfu were added and incubated stationarily at 37ºC for 30 min. After the incubation, the bacterial cells were pelleted and suspended in 2YT medium containing 100 µg carbencilin/ml and kanamycin 50 µg/ml. After incubation overnight at 37ºC, 250 rpm shaking, the bacterial cells were pelleted; the supernatant was removed and filtered with 0.45-µm filter. A one-fifths volume of PEG-NaCl (20% polyethylene glycol 8000 in 2.5 *M* NaCl) was added to the supernatant, left at 4ºC for at least 30 min, and then centrifuged (7000 x *G*, 30 min). The pelleted phages were suspended in PBS containing 20% glycerol, aliquoted and stored at -70ºC until used.

The second round of panning was carried out similarly, except that both collection of phages, *i.e* phages with NP and those with H5N1 baits in the first panning, were each panned with NP and H5N1 baits. Four different collections of phages were produced: NP-NP, NP-H5N1, H5N1-H5N1 and H5N1-NP, identified with the first and second baits. In the third round of panning, the four collections of phages were panned against NP, and phages bound to NP were eluted with 0.1 *M* glycine-HCl pH 2. A mid-log phase TG1 *E. coli* culture was infected with each of the eluted phages and plated on 2YT agar containing 100 µg/ml carbencillin and 2% glucose. Individual colony, 10 – 15 colonies per collection of phages, was picked up randomly and grown on 2 ml 2YT medium. At mid-log phase, the scFv phages were rescued with helper phage. The reactivity of scFv-phages was determined with a phage ELISA.

Phage ELISA

A 96-well plate (maxisorp, Nunc Inc.) was coated with 50 ng/well NP at 4ºC overnight. After 2-hr blocking with 2% BSA, the third panned phages were added, approximately 10^{11} pfu/ well, and incubated at 37ºC, 250 rpm for 2 hr. After 5 times washings with PBST, rabbit anti $M_{13}O_7$ phage, diluted 1 : 2000 in PBS containing 5% normal chicken serum was added, and incubated at 37ºC, 250 rpm for 2 hr. After 5 times washings with PBST, substrate and ABTS were added and optical density (A_{420}) were measured.

Phagemids were isolated from TG1 *E. coli* containing the strongest reactivity of phages using a commercial kit (QIAprep mini prep kit, Qiagen). The isolated phagemid were kept at -20ºC until use.

Transformation and clone selection

The competent cells were prepared according to a previous methods (CHUNG et al. 1989). Briefly, HB2151 *E. coli*, at early log-phase in LB broth (A_{600} = 0.35) was pelleted (1000 x G, 10 min). The pellet was suspended in transformation solution (10% polyethylene glycol 8000, 5% DMSO and 50 m*M* $MgCl_2$), one-tenth of its original volume). The cell suspension was aliquoted in 100 µl tube and kept in -80ºC until used.

For transformation, 1 ng or 0.1 ng phagemid in 1 µl volume was added to the 100 µl competent cells and incubated at 4ºC for 30 min. After adding 0.9 ml LB broth, the suspension was incubated at 37ºC, 250 rpm for 1 hr, then plated in LB agar containing 100 µg/ml carbencillin. After incubation overnight at 37ºC, individual colony, selected randomly, was touched lightly with a toothpick and suspended in 0.5 ml 2YT broth containing 100 µg/ml carbencillin and 2% glucose. For scFv expression, 10 µl of the bacterial suspension is added to fresh 2YT broth containing 100 µg/ml carbencillin and 0.5 % glucose ($2YT_{-carb-glu}$) and incubated at 37ºC, 250 rpm. At mid-log phase growth (A_{600} = 0.5), 0.1 m*M* IPTG was added and the cultures were incubated at 30ºC, 250 rpm. Following overnight incubation, the bacterial cells were pelleted, and 100 µL supernatant was added to a 96-well micro-titration plate that had been coated previously with 20 ng NP/ well. After 2 hr incubation, the plate was washed 5 times with PBST, 100 µl anti-human 2Fab (Abcam) diluted 1: 100 was added and incubated 2 hr. After washing 5 times, substrate and ABTS was added, and OD was measured after 15 and 30 minutes. Clones producing the highest OD were selected for further production of NP-specific scFv.

Expression and purification of scFv

Selected transformed *E. coli* were grown in 2YT-carb-glu to mid-log phase, and incubated overnight at 30ºC after induction with 0.1 m*M* IPTG. The bacterial cells were pelleted (8000 x G, 4ºC, 20 min) and the supernatant was removed. Purification of scFv was carried out using a nickel column chromatography (HisTrap HP, GE Healthcare life science) on a chromatographic purification system, *Acta start* (GE Healthcare life science). The eluted proteins from the column were desalted and concentrated using a 10-kDa-cut-off centriprep (Amicon). The purity was checked with a routine sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS PAGE).

The scFv antibody and reactivity of scFv against nucleoprotein and H5N1 virus

Rabbits were immunized with 1 mg of the purified NP-scFv intramuscularly using Quil A as adjuvant. Booster immunizations were given in a 4 weeks interval. Immune response was monitored using an ELISA with purified scFv as the coating antigen. Two weeks after the last immunization, the rabbits were anaesthetized and bled to death. The sera were aliquoted and stored at -20ºC.

An ELISA and dot blot were used to analyze reactivity of scFv against NP or delipidated H5N1. The plate was coated with NP (20 ng/well) or delipidated H5N1 (≈ 200 EID_{50}/ well). After blocking with 1 % BSA, purified scFv was added and incubated for 2 hr. After washing 5 times with PBST, the rabbit-anti-scFv at 1: 500 dilution was added and incubated 2hr. After washing 5 times with PBST, substrate and ABTS were added, and optical density was measured after 15 min.

For dot-blot assay, 3 µl suspension containing either 30 ng NP or 100 EID_{50} delipidated H5N1 virus were spotted onto a nitrocellulose strip. After blocking with 1% BSA, purified scFv was added and incubated for 2 hr. After washing 5 times with PBST, rabbit-anti-scFv serum at 1: 500 dilution was added and incubated 2hr. After washing 5 times with PBST, the membrane strip was developed in substrate and chromogenic 3'-diaminobenzidine (DAB).

RESULTS AND DISCUSSION

The present study successfully isolated scFv monoclonal antibody that recognized recombinant NP from influenza H1N1 and H5N1 influenza virion. The capacity of recognizing both influenza-virus subtypes is attributed to the panning strategy used in this study, which is alternating NP and H5N1 as baits, and elution of bound phages with anti-H5N1 serum.

Delipidation of H5N1 virion, as carried out in this study, was supposed to increase its immobilization on the polystyrene surface of the immunotube and plate. Tight binding of the virion to the immunotube was necessary to withstand intensive (12 times) washings during the panning process. Previously, when NP was used as bait singly or as the only bait, the isolated scFv recognized the NP but not H5N1 virion. As far as we were aware, this panning approach together with the elution with the anti-serum had not been used previously.

After the first panning, the reactivity of phage to NP was still unapparent (Figure 1). The reactivity was similar to that of the control, M13O7 helper phage. After the second panning, either the first with NP and the second with NP or H5N1 virus, or the first with H5N1 virus and the second with NP, however, the reactivity increased impressively. For unknown reason, however, the reactivity of the phage after panning twice with H5N1 virus remained undetected. Reactivity of phage after the third panning on NP is presented on Table 1. The phages, which were rescued from randomly selected colonies of TG1 *E. coli*, had comparable reactivity. The reactivity, as expressed in ELISA OD's, were about 4 times as higher as that of M13O7 helper phage control.

For the production of soluble anti-scFv antibody, the phagemid from the suppressive TG1 *E. coli* was isolated and expressed in a suppressive *E. coli* strain, HB2151. Only in this non-suppressive strain does the amber stop codon (TAG), which placed as the last codon of scFv, function as a proper stop codon.

Considerable amount of phagemid, 7.8 and 3.8 µg, respectively, was isolated from two colonies of TG1-*E. coli* harboring phage with high reactivity to the NP. When competent HB2151 *E. coli* cells were transformed with the NP-scFv-phagemid, they produced a high number of colonies on carbencillin-LB-agar plates due to the presence amphicilin-resistant gene in the phagemid. Ninety colonies were randomly selected and the capacity of each colony to expressed scFv recognizing NP is presented in Table 2. One colony (#47) expressing scFv with the highest reactivity was chosen for further scFv purification.

In *E. coli* proteins are synthesized in the cytoplasm, some of which, however, may be translocated into the periplasmic compartment, and proteins accumulated in the periplasmic compartment may, in turn, leak out into the culture medium (Kipriyanov et al. 1997). The amount of proteins leaking into the medium depend on the primary structure or amino acid sequence of the protein, stability of membrane, composition of the media and duration of incubation (Bäcklund 2008). In regard to protein purification, purification of expressed protein from the culture media is easier than that from the periplasmic or cytoplasmic spaces

In addition to the antibiotic resistance gene, the phagemid used in this study also equipped with the ompA leader sequence, which translocated the newly synthesized scFv from cytoplasm to the periplasmic compartment where the disulphide bonds stabilizing the molecule were formed. Also, to ease protein purification, the phagemid was equipped with a DNA sequence encoding poly histidin as a tag at the C-terminal of the scFv protein.

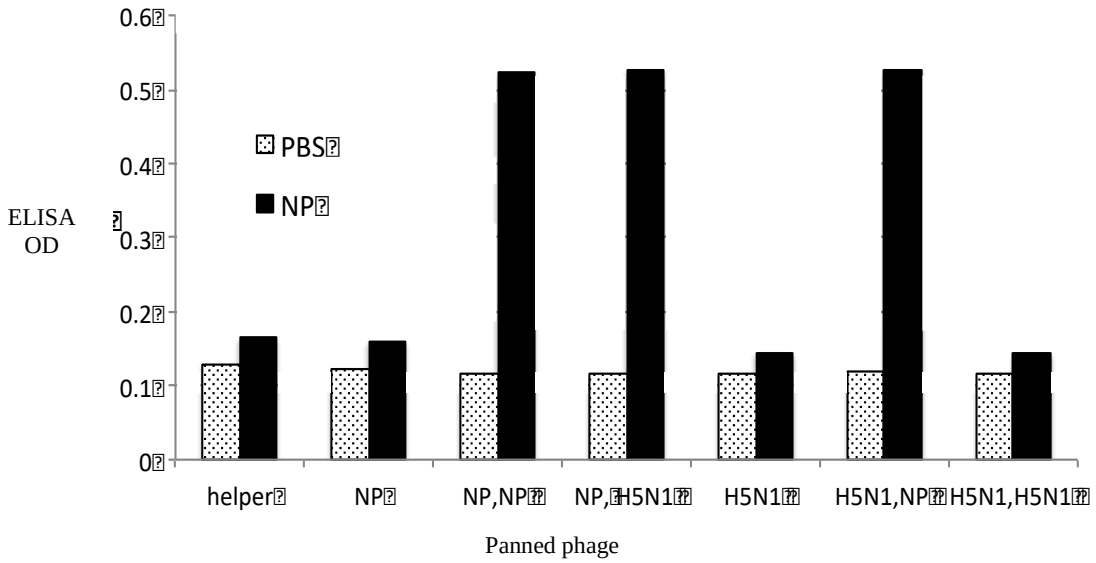


Figure 1. Reactivity of phages to NP or H5N1 after the first or second panning. The symbol NP or H5N1 on the x-axis denotes the bait used in the panning. Two codes separated by a comma, *eg.* NP, H5N1, represent baits used in the first and second panning, respectively. The symbol "Helper" represents M13O7 helper phage used as a control.

Table 1. Reactivity of phage after the third panning on NP bait, propagated and rescued from randomly selected colonies of TG1 *E. coli*

Phage H5N1-NP		Phage NP-NP		Phage NP-H5N1		Helper phage
Colony	ELISA OD	Colony	ELISA OD	Colony	ELISA OD	ELISA OD
5A	1.4165	6A	1.5435	7A	1.6195	0.413
5B*	**1.347**	6B	0.913	7B	1.5595	
5C*	**1.636**	6C	1.3795	7C	1.337	
5D	1.585	6D	1.0225	7D	1.461	
5E	1.3265	6E	1.5665	7E	1.384	
5F	1.5985	6F	1.512	7F	1.453	
5G	1.666	6G	1.616	7G	1.479	
5H	1.565	6H	1.5535	7H	1.471	
5I	1.6155	6I	1.429	7I	1.492	
5J	1.326	6J	1.1265	7J	1.181	
5K	1.5345	6K	1.4365			
5L	1.647	6L	1.491			
5M	1.5515	6M	1.439			
5N	1.3115	6N	1.474			
5O	1.5675	6O	1.4735			

(*) colonies from which phagemid was isolated and used to transform competent HB2151 *E. coli*

Table 2. Reactivity of supernatants from randomly selected 90 colonies of HB2151 E.coli transformed with phagemid that had undergone thrice panning with NP and H5N1 virus (colonies# 5B and 5C, Table 1)

Col #	OD	Col #	OD	Col #	OD	Col #	OD	Col #	OD	Col #	OD
1	0.408	16	0.394	31	0.372	46	0.363	61	0.361	76	0.384
2	0.427	17	0.457	32	0.41	47*	0.604	62	0.32	77	0.364
3	0.366	18	0.391	33	0.428	48	0.424	63	0.37	78	0.376
4	0.356	19	0.361	34	0.389	49*	0.451	64	0.397	79	0.381
5	0.34	20	0.371	35	0.355	50	0.431	65	0.411	80	0.484
6	0.349	21	0.371	36	0.349	51	0.408	66	0.378	81	0.418
7	0.383	22	0.364	37	0.365	52	0.392	67	0.359	82	0.392
8	0.398	23	0.395	38	0.376	53	0.347	68	0.37	83	0.331
9	0.387	24	0.418	39	0.375	54	0.34	69	0.377	84	0.366
10	0.39	25	0.396	40	0.401	55	0.374	70	0.331	85	0.358
11	0.354	26	0.368	41	0.428	56	0.412	71	0.369	86	0.372
12	0.36	27	0.351	42	0.38	57	0.396	72	0.363	87	0.364
13	0.374	28	0.307	43	0.36	58	0.355	73	0.391	88	0.37
14	0.386	29	0.353	44	0.381	59	0.356	74	0.395	89	0.43
15	0.38	30	0.359	45	0.372	60	0.327	75	0.375	90	0.42

Expression of anti-NP scFv antibody in the present study revealed that the amount of scFv recovered from culture supernatant was larger than that from periplasmic compartment. Based on SDS PAGE, the major protein eluted from the nickel-ion-affinity chromatography had a molecular weight of about 27 kDa, similar to that of expected scFv (Figure 2). This protein was highly immunogenic, as a high titre against the NP-scFv was obtained after the fifth immunization of rabbits. At 1: 1600 dilutions, the antiserum recognized the scFv coated on micro titre plate at a concentration of 44 ng/ml. At lower dilutions (1 : 200), it recognized at a concentration of 5 ng/ml.

The purified scFv proved to recognized NP and H5N1 both in dot blot and ELISA (Figure 3 and 4). In dot blot experiment, the bindings of scFv to NP and to H5N1 virion were probed by the rabbit anti scFv serum. This experiment also proved the specificity of the antibody, as no signal was observed when the binding was probed with normal or pre-vaccinated serum (Figure 3). Results of this dot blot experiment were in agreement with those of ELISA. The rabbit anti-scFv serum affirmed the binding of purified scFv to NP or to H5N1 virion. The binding scFv to NP or scFv to H5N1 that was probed with the rabbit anti-scFv serum prompted ELISA ODs that were about five times higher than those probed with negative serum. The binding scFv to NP or scFv to H5N1 that was probed with the rabbit anti-scFv serum produced ELISA ODs that were about five times higher than that probed with negative serum. Non-specific bindings between the negative serum with scFv, NP or H5N1 were negligible. For unknown reason, however, there was some non-specific binding between the anti-scFv serum with NP or H5N1 (Figure 4).

Comparable approach to the present study had been used by previous studies in an attempt to isolate scFv recognizing parvalbumin allergen from various species of fish (Bublin et al. 2015). For that purpose, the group carried out three sequential panning on cod, carp and rainbow-trout parvalbumins, respectively.

One of the most common problems in scFv production is the low yield of functional scFv that can be purified from the prokaryotic expression system. The causes of the problem include inhibition of culture growth by toxic effect of the expressed scFv, formation of insoluble aggregates in the periplasmic compartment, and plasmid instability (Mergulhao et al. 2005; Rippmann et al. 1998). To be functional, a scFv required a post-translational processing, that is the formation of disulphide bridges (Montoliu-Gaya et al. 2017; Ramm et al. 1999). Formation of disulphide bonds in prokaryotic cells is taken place only in the periplasmic compartment because only in this compartment the oxidative environment and required enzymes are available (Eser et al. 2009; Makrides 1996).

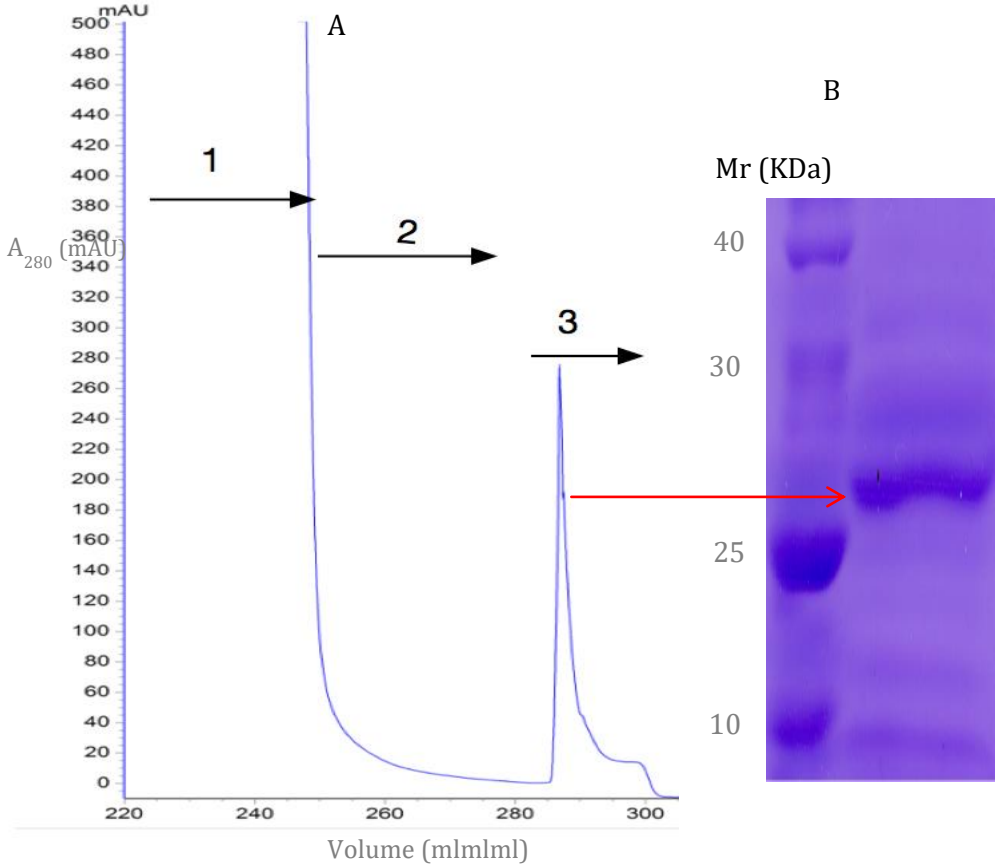


Figure 2. A: Purification scFv from culture supernatant of HB2151 *E. coli* transformed with anti-NP phagemid. Culture supernatant was loading into a 5-ml-affinity column (HisTrap HP®) (1), unbound proteins were washed off from the column (2), bound protein (scFv) was eluted with imidazole solution (3).
B: Coomasie-blue stained SDS PAGE of protein eluted from affinity column after desalting and concentration. The elution contains a single protein with a ≈ 27kDa size, similar to that of scFv (red arrow).

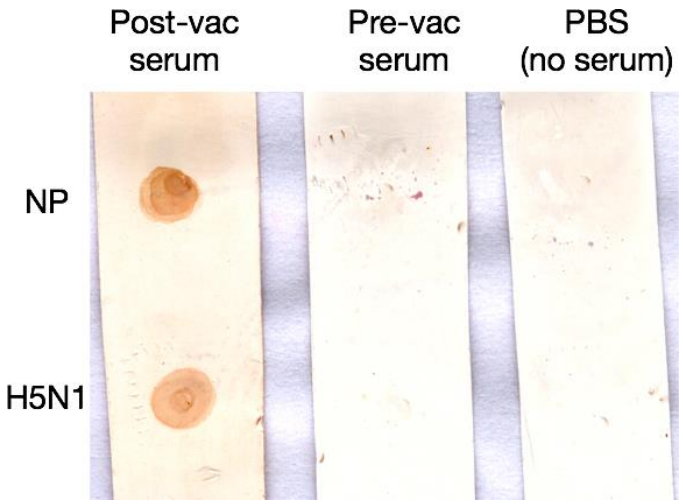


Figure 3. Reactivity of scFv against H1N1 nucleoprotein (NP) and H5N1 virion by dot blot. The NP (1 µg) or delipidated H5N1 (≈100 EID_{50}) is spotted onto nitrocellulose strips, the NP-scFv was added and probed with rabbit anti-scFv serum (post-vac serum), normal rabbit serum (pre-vac serum) or PBS (control).

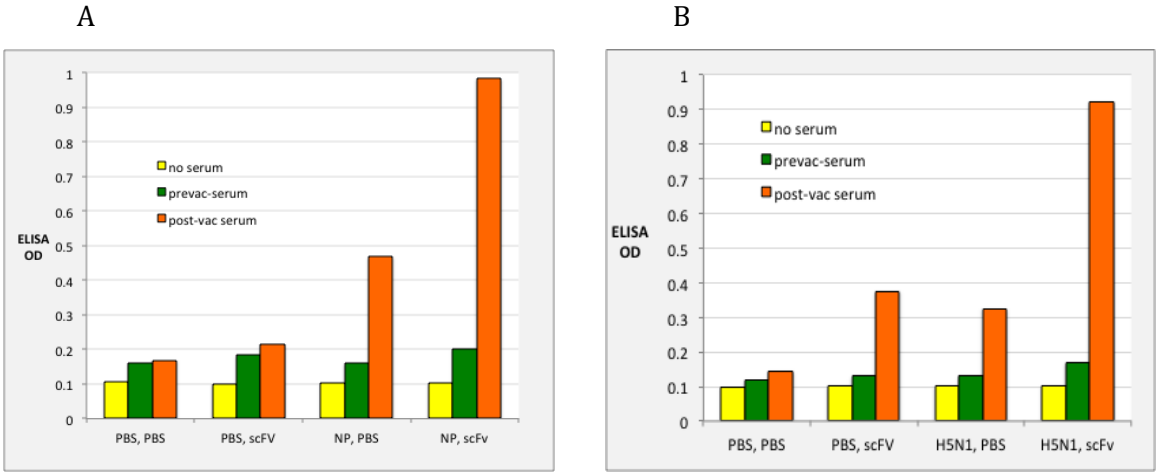


Figure 4. Reactivity of scFv against H1N1 nucleoprotein (NP) (A) and against H5N1 virion (B) by ELISA. Micro-titer plate wells coated with NP, H5N1 or PBS (control) were added either scFv or PBS (control). After washings, rabbit anti-NP-scFv serum (post-vac serum), normal rabbit serum (pre-vac serum) or PBS (control) were added.

Although the functional scFv accumulated in the periplasmic space, some of them may leak out into the culture medium (Kipriyanov et al. 1997). In this study, the amount of scFv purified from the culture medium was found to be higher than that from the periplasmic compartment isolated by the cold osmotic shock method (Neu & Heppel 1965). For this reason, isolation of scFv was only carried out from this compartment. In addition, isolation of proteins from the culture medium is easier that that from periplasmic compartment (Kipriyanov et al. 1997).

In the present study, about 1.2 mg of anti-NP-scFv antibody per litre culture was obtained. This yield is considered to be high as compared to previous studies; 0.59 mg/L (Mesgari-Shadi & Sarrafzadeh 2017) or 0.1 mg/L (Kipriyanov et al. 1997). Eukaryotic cells are apparently more efficient than prokaryotic cells in expressing functional scFv. An expression of scFv in mammalian HEK293T cells was reported to obtain a yield of 12.87 to 33.56 mg/L culture (Pipattanaboon et al. 2017). Yeast (*Pichia pastoris*) expression system was reported to obtain even higher yield, 100 mg/L of pure and functional rabbit anti-rhLIF scFv antibody (Ridder et al. 1995).

Various attempts to increase scFv yields in prokaryotic expression system have been made. Kipriyanov and group reported that addition of sucrose to the medium resulted in the yield of 16.5 mg/L scFv or 80- 150 fold higher than that without sucrose addition (Kipriyanov et al. 1997). Similar results were also obtained by others (Mesgari-Shadi & Sarrafzadeh 2017; Sawyer et al. 1994). In our study, however, no increase in the yield was observed when sucrose was added to the culture media. This means that addition of sucrose to the culture does not always increase the functional scFv yield. As a matter of fact, Sina and group reported that addition of sucrose to the culture media even suppressed the expression of scFv (Sina et al. 2015). The cause of the differences in the response to the sucrose is unknown but it might be related to the primary sequence of the scFv (Takkinen et al. 1991). In addition to the primary sequence of the scFv, there are some other factors may affect the expression of scFv in prokaryotic cells including duration, temperature, aeration and gene induction. Each of those factors needs to be optimalisized for every scFv, which is time consuming when carried out in flask cultures. However, a simple optimaliation can be carried out in using a fermentor by sequential simplex optimization method (Intachai et al. 2015).

CONCLUSION

A scFv-monoclonal antibody recognizing nucleoprotein of influenza virus was isolated by panning a commercial-influenza-A- focused- scFv library. Panning with the alternating H1N1 NP and H5N1 virion, and elution with H5N1 antiserum assure the isolated mAb recognizes multiple, if not all, subtype of influenza-A viruses. The anti NP-scFv antibody was purified to homogeneity using an affinity chromatography. Rabbits immunized with this purified NP-scFv produced specific antibody that recognized NP-scFv even in a very low concentration in immunoassays. The immunoassays carried out in this study suggest that the NP-scFv mAb and the rabbit anti NP-scFv can be use in developing point-of-care diagnostic tools for Avian Influenza.

REFERENCES

Bäcklund E. 2008. Growth rate control of periplasmic product retention in *Escherichia coli* (Thesis). [Stockholm (UK)]: Royal Institute of Technology.

Bublin M, Kostadinova M, Fuchs JE, Ackerbauer D, Moraes AH, Almeida FC, Lengger N, Hafner C, Ebner C, Radauer C, Liedl KR, Valente AP, Breiteneder H. 2015. A cross-reactive human single-chain antibody for detection of Major Fish Allergens, Parvalbumins, and identification of a Major IgE-Binding epitope. PLoS One. 10:e0142625.

Cham BE, Knowles BR. 1976. A solvent system for delipidation of plasma or serum without protein precipitation. J Lipid Res. 17:176-181.

Chung CT, Niemela SL, Miller RH. 1989. One-step preparation of competent *Escherichia coli*: Transformation and storage of bacterial cells in the same solution. Proc Natl Acad Sci. 86:2172-2175.

Clackson T, Hoogenboom HR, Griffiths AD, Winter G. 1991. Making antibody fragments using phage display libraries. Nature. 352:624-628.

Eser M, Masip L, Kadokura H, Georgiou G, Beckwith J. 2009. Disulfide bond formation by exported glutaredoxin indicates glutathione's presence in the E. coli periplasm. Proc Natl Acad Sci. 106:1572-1577.

[FAO] Food Agriculture Association. 2013. Lessons from HPAI-A technical stocktaking of coutputs, outcomes, best practices and lessons learned from the fight against highly pathogenic avian influenza in Asia 2005-2011. Rome (Italy): Food Agriculture Association.

Hoogenboom HR, de Bruine AP, Hufton SE, Hoet RM, Arends JW, Roovers RC. 1998. Antibody phage display technology and its applications. Immunotechnology. 4:1-20.

Intachai K, Singboottra P, Leksawasdi N, Kasinrerk W, Tayapiwatana C, Butr-Indr B. 2015. Enhanced production of functional extracellular single chain variable fragment against HIV-1 matrix protein from *Escherichia coli* by sequential simplex optimization. Prep Biochem Biotechnol. 45:56-68.

Kipriyanov SM, Moldenhauer G, Little M. 1997. High level production of soluble single chain antibodies in small-scale *Escherichia coli* cultures. J Immunol Methods. 200:69-77.

Kohler G, Milstein C. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 256:495-497.

Liu JK. 2014. The history of monoclonal antibody development - Progress, remaining challenges and future innovations. Ann Med Surg. 3:113-116.

Makrides SC. 1996. Strategies for achieving high-level expression of genes in *Escherichia coli*. Microbiol Rev. 60:512-538.

Mergulhao FJ, Summers DK, Monteiro GA. 2005. Recombinant protein secretion in *Escherichia coli*. Biotechnol Adv. 23:177-202.

Mesgari-Shadi A, Sarrafzadeh MH. 2017. Osmotic conditions could promote scFv antibody production in the *Escherichia coli* HB2151. Bioimpacts. 7:199-206.

Montoliu-Gaya L, Martinez JC, Villegas S. 2017. Understanding the contribution of disulfide bridges to the folding and misfolding of an anti-Abeta scFv. Protein Sci. 26:1138-1149.

Neu HC, Heppel LA. 1965. The Release of Enzymes from *Escherichia coli* by Osmotic Shock and during the Formation of Spheroplasts. J Biol Chem. 240:3685-3692.

Nissim A, Hoogenboom HR, Tomlinson IM, Flynn G, Midgley C, Lane D, Winter G. 1994. Antibody fragments from a 'single pot' phage display library as immunochemical reagents. EMBO J. 13:692-698.

Pipattanaboon C, Boonha K, Benjathummarak S, Pitaksajjakul P, Ramasoota P. 2017. Construction and expression of H5N1 influenza virus hemagglutinin-specific scFv-Fc monoclonal antibodies in HEK293T cells. Southeast Asian J Trop Med Public Health. 48:45-55.

Ramm K, Gehrig P, Pluckthun A. 1999. Removal of the conserved disulfide bridges from the scFv fragment of an antibody: effects on folding kinetics and aggregation. J Mol Biol. 290:535-546.

Ridder R, Schmitz R, Legay F, Gram H. 1995. Generation of rabbit monoclonal antibody fragments from a combinatorial phage display library and their production in the yeast *Pichia pastoris*. Biotechnology. 13:255-260.

Rippmann JF, Klein M, Hoischen C, Brocks B, Rettig WJ, Gumpert J, Pfizenmaier K, Mattes R, Moosmayer D. 1998. Procaryotic expression of single-chain variable-fragment (scFv) antibodies: secretion in L-form cells of *Proteus mirabilis* leads to active product and overcomes the limitations of periplasmic expression in *Escherichia coli*. Appl Environ Microbiol. 64:4862-4869.

Sawyer JR, Schlom J, Kashmiri SV. 1994. The effects of induction conditions on production of a soluble anti-tumor sFv in *Escherichia coli*. Protein Eng. 7:1401-1406.

Shalit I, McKee PA, Beauchamp H, Waner JL. 1985. Comparison of polyclonal antiserum versus monoclonal antibodies for the rapid diagnosis of influenza A virus infections by immunofluorescence in clinical specimens. J Clin Microbiol. 22:877-879.

Sina M, Farajzadeh D, Dastmalchi S. 2015. Effects of Environmental Factors on Soluble Expression of a Humanized Anti-TNF-alpha scFv Antibody in *Escherichia coli*. Adv Pharm Bull. 5:455-461.

Smith GP, Petrenko VA. 1997. Phage display. Chem Rev. 97:391-410.

Takkinen K, Laukkanen ML, Sizmann D, Alfthan K, Immonen T, Vanne L, Kaartinen M, Knowles JK, Teeri TT. 1991. An active single-chain antibody containing a cellulase linker domain is secreted by *Escherichia coli*. Protein Eng. 4:837-841.

Tarigan S. 2016. Peranan *point-of-care test* dalam pengendalian *highly pathogenic Avian Influenza* di Indonesia. Wartazoa. 26:39-50.

Tarigan S, Indriani R, Durr PA, Ignjatovic J. 2015. Characterization of the M2e antibody response following highly pathogenic H5N1 avian influenza virus infection and reliability of M2e ELISA for identifying infected among vaccinated chickens. Avian Pathol. 44:259-268.

Thompson J, Pope T, Tung JS, Chan C, Hollis G, Mark G, Johnson KS. 1996. Affinity maturation of a high-affinity human monoclonal antibody against the third hypervariable loop of human immunodeficiency virus: use of phage display to improve affinity and broaden strain reactivity. J Mol Biol. 256:77-88.

Evaluation of Surra Treatment Strategies for Horses and Buffaloes in East Sumba District, Nusa Tenggara Timur Province of Indonesia (2010 – 2016)

Dewi RS[1,2], Wardhana AH[3], Soejoedono RD[4], Mulatsih S[5]

[1] *Indonesian Agricultural Quarantine Agency, Ministry of Agricultural of Indonesia, Jl Harsono RM Ragunan, Jakarta*
[2] *Veterinary Public Health, IPB Postgraduate School, IPB University, Kampus IPB Darmaga Bogor*
[3] *Departement of Parasitology, Indonesia Research Center for Veterinary Sciences, Jl R.E Martadinata No. 30, Bogor*
[4] *Department of Animal Disease and Veterinary Public Health, IPB University, Jl. Agatis, Kampus IPB Darmaga, Bogor*
[5] *Departement of Economic Science, IPB University, Jl. Babakan, Kampus IPB Darmaga, Bogor*

(received 25-09-2018; revised 18-03-2019; accepted 18-03-2019)

ABSTRACT

Dewi RS, Wardhana AH, Soejoedono RD, Mulatsih S. 2019. Evaluasi strategi penanganan surra untuk kuda dan kerbau di Kabupaten Sumba Timur, Provinsi Nusa Tenggara Timur Indonesia (2010 – 2016). JITV 24(1): 39-48. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1864

Surra adalah penyakit yang menyerang ternak dan disebabkan oleh protozoa berflagella, *Trypanosoma evansi*. Kepulauan Indonesia telah dilaporkan sebagai negara endemik penyakit ini, kecuali Pulau Sumba. Namun, wabah Surra terjadi di pulau ini pada tahun 2010 karena lalu lintas ternak dari pulau tetangga, Sumbawa. Hal ini menyebabkan kematian yang tinggi pada ternak, khususnya kuda dan kerbau. Tujuan penelitian ini adalah untuk mengevaluasi efektivitas strategi pengobatan Surrra di Kabupaten Sumba Timur dari tahun 2010 – 2016 and untuk memprediksi kejadian kasus Surra pada 12 bulan mendatang (*forecast*). Strategi pengobatan Surra di Kabupaten Sumba Timur dibagi menjadi dua periode, yaitu periode I pada tahun 2010-2011 menggunakan isometamedium sebagai obat tunggal dan periode II pada tahun 2012 – 2016 menggunakan kombinasi antara *diminazene aceturate* sebagai obat penyembuhan and *isometamedium* sebagai obat pencegahan. Data untuk studi ini diperoleh dari Dinas Peternakan Kabupaten Sumba Timur dari 2010 – 2016 ketika wabah Surra terjadi. Efektivitas kedua strategi pengobatan tersebut dianalisis menggunakan uji proporsional. Hasil studi membuktikan bahwa morbiditas dan mortalitas kuda dan kerbau pada periode I lebih tinggi daripada periode II. Strategi pengobatan pada periode II mampu menurunkan proporsi morbiditas pada kuda dan kerbau, masing-masing menjadi 1,44% dan 0,66%. Demikian pula, proporsi mortalitas pada periode II lebih kecil daripada periode I, yaitu dari 3,79% menjadi 1,30% pada kuda dan 2.8% menjadi 0,55% pada kerbau. Berdasarkan analisis *forecasting* menggunakan metode "*control program with decomposition*" untuk 12 bulan mendatang membuktikan bahwa strategi pengobatan periode II mengurangi kejadian dan kematian ternak karena Surra. Strategi pengobatan menggunkan kombinasi antara *isometamedium* dan *diminazene aceturate* in Sumba Timur lebih efektif daripada menggunakan *isometamedium* sebagai obat tunggal.

Kata Kunci: Surra, *Trypanosoma evansi*, Pengobatan, Trypanosidal, Sumba Timur

ABSTRACT

Dewi RS, Wardhana AH, Soejoedono RD, Mulatsih S. 2019. Evaluation of surra treatment strategies for horses and buffaloes in East Sumba District, Nusa Tenggara Timur Province of Indonesia (2010 – 2016). JITV 24(1): 39-48. DOI: http://dx.doi.org/10.14334/jitv.v24i1.1864

Surra is a disease attacking livestock caused by a flagellated protozoan, *Trypanosoma evansi*. Indonesia archipelago is reported as an endemic country of the disease, except Sumba Island. However, Surra outbreak occurred in this Island in 2010 due to livestock movement from the neighbour island, Sumbawa. It generated high mortality in livestock, particularly in horses and buffaloes. The aim of this study was to evaluate the effectiveness of Surra treatment strategies in East Sumba District from 2010-2016 and to estimate the incidence of Surra in the next few months (forecast). The treatment strategy of Surra in East Sumba was divided into two periods namely: the first period in 2010-2011 using Isomethamedium as the single drug (period I) and the second period in 2012 - 2016 using a combination between diminazene aceturate as curative and isomethamedium as a prophylactic drug (period II). All data in the present study was obtained from the local livestock agency of East Sumba District from 2010 – 2016 when Surra outbreak occurred. The effectiveness of those two treatment strategies was compared using the proportion test. The results demonstrated that morbidity and mortality of horses and buffaloes were significantly greater in the period I (2010-2011) compared to period II (2012-2016). The treatment strategy in the period II was able to decrease the proportion of morbidity in horses and buffaloes for 1.44% and 0.66%, respectively. Likewise, the proportion of mortality in period II was also less than the period I from 3.79% to 1.30% for horses and from 2.80% to 0.55% for buffaloes. Based on forecasting study analysis using the control program projected with decomposition method for the next 12 months demonstrated that the treatment strategy in the period II could reduce the incidence and death of livestock by Surra. The treatment strategy using a combination between isometamedium and diminazene aceturate in East Sumba District might be more effective compared to using isometamedium alone.

Key Words: Surra, *Trypanosoma evansi*, Treatment, Trypanocidal, East Sumba

INTRODUCTION

Trypanosoma evansi (*T. evansi*) is a haemo – protozoan of the genus Trypanosoma as the causative agent of Surra. The parasite is geographically distributed in the tropical and subtropical areas such as Central and South America, Africa, Middle East and Asia, including Indonesia and attacks multispecies animals characterized by polymorphism of its clinical symptoms (Camoin et al. 2017; Aregawi et al. 2019). Surra is transmitted mechanically from an infected animal to another susceptible animal by haematophagous flies particularly Tabanus spp, Stomoxys spp, Haematopota spp, Lyperosia spp and Chrysops spp. (OIE 2012; Desquesnes et al. 2013) leading to reduce productivity, high mortality of host, various neurological disorders and major economic losses (Ponnudurai et al. 2015; Tehseen et al. 2017). According to Sivajothi et al. (2014) that the incidence and the severity of Surra depend on the strain of the parasite (level of virulence) as well as the species of host affected.

The first incidence of Surra in Indonesia occurred in Semarang of Central Java Province attacking horse herds. Furthermore, some outbreaks of Surra were found in cattle and water buffaloes in East Java Province (Payne et al. 1991). The disease was rapidly widespread throughout the archipelago and presented on all the main islands (Luckins 1998), excluding Sumba Island until 2009. However, Sumba Island was introduced to *T. evansi* in 2010 due to livestock movement from Sumbawa Island (endemic area of Surra). It caused severe Surra outbreak causing high mortality in horses and buffaloes in 2010 – 2012.

The largest district possessing large savannah which is traditionally major site of livestock farming with large herds of cattle, horses, buffaloes and other animals. In addition, the population of livestock in East Sumba District relatively higher compared to other regions. It plays a role as one of the main supplier of livestock for other area of Indonesia. Accordingly, Sumba Island is one of the national center for livestock development in Indonesia and Surra became a serious threat which potentially devastated livestock population in Indonesia, particularly in Sumba.

In order to control Surra, the Regent of East Sumba issued Decree of Regent No: 185 / Disnak.524.3 / 570 / VII / 2010 and Instruction of Regent No: 147 of 2010 in July on the emergency response from Surra threat in East Sumba district in 2010. Singh & Singla (2015) proposed that there were three steps to prevent Surra transmission in the field e.g. controlling of the parasite, controlling of the vector (biting flies) and using of innate resistance of the host to the effect of the infection. At the beginning of Surra outbreak in Sumba, those steps could not be employed because Sumba was previously region free from Surra so that all livestock did not have innate immune. When Surra was taken out from the list of strategic animal diseases in Indonesia, the availability of Surra drug was very few, including in East Sumba. The local vets and farmers faced difficulties to deal with the disease. Later, the government supported isometamedium with a limited number in 2010 -2011 and provided diminazene aceturate in 2012. They employed two treatment strategies to cure livestock from *T. evansi* infection. Unfortunately, they did not have a proper program of vector control. As a result, the mortality level of horses and buffaloes increased dramatically.

To date, no comprehensive assessment of the drug effectiveness has been carried out for Surra in livestock in Indonesia, particularly in East Sumba District. Therefore, this study was designed to evaluate Surra treatment strategies applied in East Sumba during and after Surra outbreak, including predicting the incidence of Surra in the future using forecasting study involving retrieval of historical data (2010-2016) (Heizer & Render 2009).

MATERIALS AND METHODS

Samples and locations

The present study was conducted in East Sumba District, East Nusa Tenggara Province. The district consists of 22 sub-districts (Figure 1). Time series data of Surra from 2010 – 2016 was obtained from the local livestock agency. They were mortality, morbidity, the population at risk, treatments and laboratory observation data. In addition, an interview was conducted to some local vets, staffs at the local livestock agency and farmers to support and confirm the data. The number of the infected animal from 2010-2016 can be seen in Table 1. The infected livestock was confirmed by both the clinical symptom and laboratory test (blood smear). The local livestock agency of East Sumba collected and recorded the data from farmer‘s reports, surveillance and monitoring results.

Treatment periods

There were two periods of Surra treatment in East Sumba District. The difference between the first and the second periods was the drug used and the strategy of treatment management employed (Figure 1).

Period 1 was the treatment strategy applied when the first Surra outbreak occurred in East Sumba District in 2010 – 2011. Isometamedium was the only available drug of Surra at the time. The drug was used for both curative and preventive treatments. Due to limited drug number, the treatment was not followed by further observation and only delivered to positive cases or herd of livestock at the pen.

Period 2 was the treatment strategy of Surra carried out in 2012 – 2016. There were two drugs of Surra registered in Indonesia e.g. isometamedium and

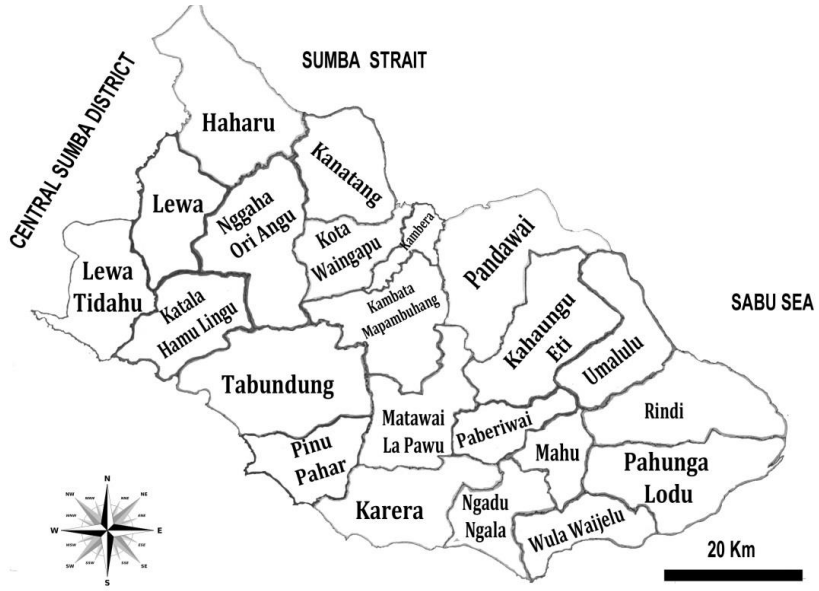


Figure 1. Map of East Sumba District with 22 sub-districts marked by name of the sub-district.

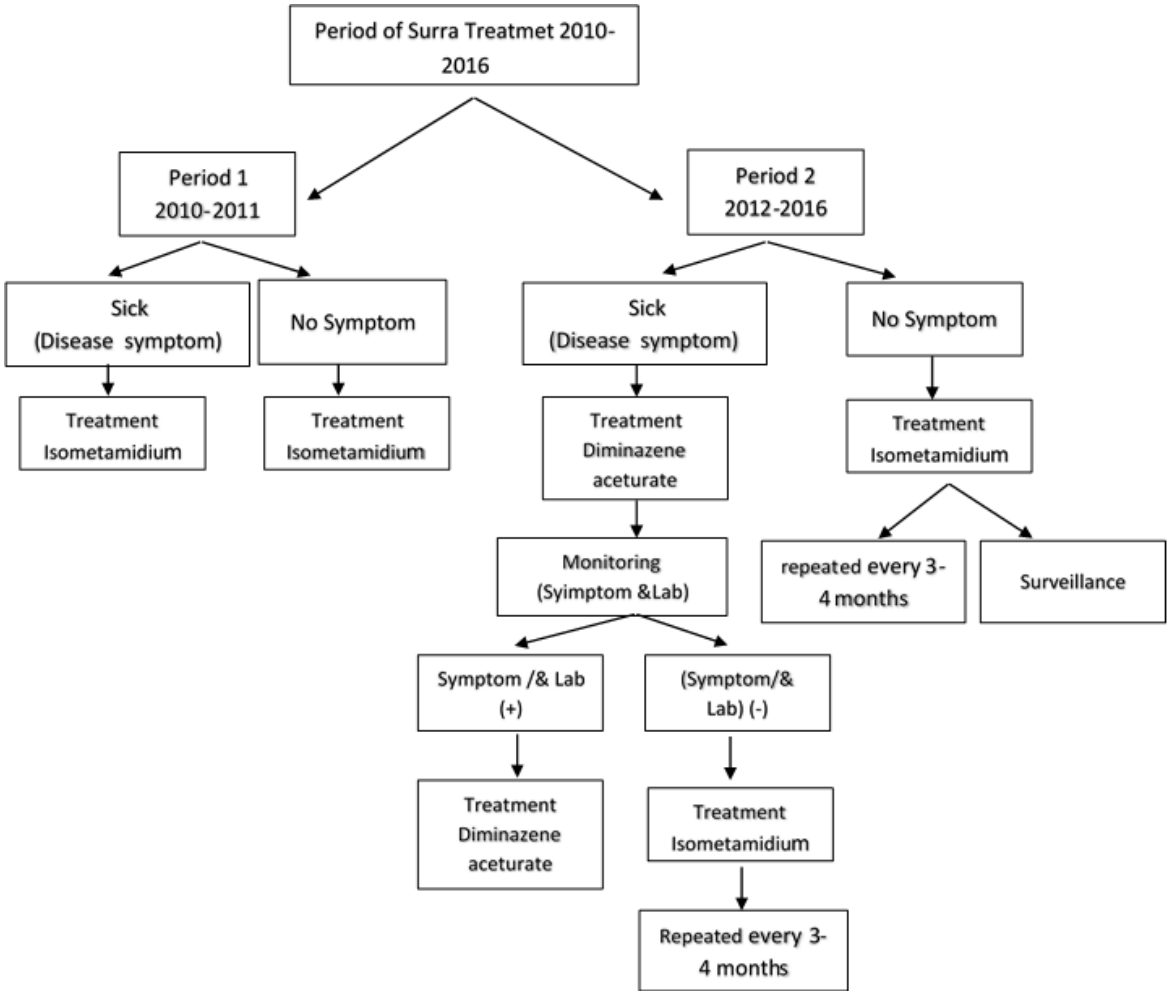


Figure 2. The Strategy of Surra treatment in East Sumba 2010-2016.

diminazene aceturate with sufficient number. The treatment management system of Surra has been compiled comprehensively using those drugs and conducted at the village level. For infected livestock, they were treated using diminazene aceturate as curative treatment and uninfected livestock (population in risk) were treated using isometamedium as a preventive treatment. Subsequently, they were observed for two weeks. If the infected livestock remained sick, they would be re-treated using diminazene aceturate, followed by second observation after two weeks. For those livestock recovered from Surra, they would be monitored regularly every 3 – 4 months and treated using isometamedium as preventive (Figure 2).

Forecasting study

Forecasting is a scientific calculation that aims to predict future conditions using data and information in the past (Harlan et al. 2018). The study was addressed to generate a prediction of Surra cases in the future based on time series data by analysis of trends. The models used in the present study were simple and ignore a number of complexities such as level of infection, number of the parasite, host heterogeneities and the population dynamics of vectors (Coen et al. 2001). Data of morbidity, mortality and fatality from 2010 – 2016 were analyzed in this study. According to Putt et al. (1988), the morbidity is defined as the proportion of affected individual in a population and the mortality is the proportion of animal dying in a population, and case fatality rate is the proportion of animal dying within affected animals.

Data analyses

Comparison of those treatment strategies analyses was performed using the proportion test consisting of the proportion of morbidity, mortality and case fatality rates. In addition, forecasting analysis with decomposition method was used to predict the incidence of Surra in the future. The model analysis selected was the method with the smallest errors and the best accuracy using minitab 16 Statistical software (Heizer & Render 2009).

RESULT AND DISCUSSION

In general, livestock raised in Sumba Island is an extensive traditional management system defined as a system of husbandry where livestock released during the day and stable at night, and or released on grazing day and night. In this way, the farmer intervention is very limited (Kapa et al. 2017). *Trypanosoma. evansi* was introduced into Sumba Island starting from Southwest Sumba, and then rapidly spread to West Sumba, Central Sumba, and East Sumba Districts (Dongga 2013). First Surra outbreak occurred in 2010 attacking horses and the second outbreak was in 2011 attacking buffaloes. It generated a major problem both in economic and social aspects (Ndiha et al. 2018). Due to the extensive traditional management system, the farmers did not realize if the number of their livestock decreased because of Surra.

There were two hypotheses explaining Sumba Island getting *T. evansi* from a neighbour endemic island (Sumbawa Island). Firstly, an infected horse came from Bima (Sumbawa Island) to Sumba Island when there was a traditional horse racing. People in Sumba dan Bima have a similar cultural approach to horse racing. There is an annual traditional horse racing in both places. If the racing is conducted in Sumba, many horses from Bima come to Sumba for joining the event. Conversely, if the racing is held in Bima, people in Sumba will transport their horse racing to Bima. This event might facilitate the spread of Surra from Bima (Sumbawa Island) to Sumba Island (Dongga 2013). Secondly, introducing Surra to Sumba was facilitated by buffaloes trading from Sumbawa to Sumba Island. The price of buffaloes in Sumbawa Island is cheaper than in Sumba Island so that many farmers or livestock trader buy and transport buffaloes from Sumbawa Island to Sumba Island.

In 2010, all villages in both Lewa and Lewa Tidahu sub-district were endemic Surra and around four sub-districts surrounded were threatened from *T. evansi* infection e.g. Nggaha Ori Angu, Katala Hamu Lingu, Kota Waingapu and Kambera. Furthermore, the disease spread to Tabundung sub-district, particularly in Praingkareha village in 2011 and distributed widely to Ngadu Ngala and Wula Waijelu sub-districts in 2012. The accumulation data of Surra from 2012 – 2016 revealed that the heights number of Surra cases was found in Kota Waingapu sub-district, followed by Wula Waijelu, Ngadu Ngala, Pohunga Lodu and Lewa. The widespread Surra among sub-districts in East Sumba was caused by livestock movement related to the traditional culture of Sumbanese called "Bellis", a tradition of dowry gift (normally livestock e.g. horses, buffaloes, cattle) from men to women in the marriages. It was believed as a primary causative source of surra distribution in East Sumba because some traditional farmers did not follow the procedure of animal inspection conducted by the staff from local livestock agency in East Sumba District.

In East Sumba district, the farmers raise horses, buffaloes and cattle together. It puts horses under severe risk because buffaloes and cattle act as a source of *T. evansi* infection (Kundu et al. 2013). Sumbria et al. (2017) stated that open grazing practices in equine might increase the risk of the infection. Advanced management and disease control program is fundamentally needed to reduce the chance of *T. evansi* infection in equines. Nurulaini et al. (2013) strongly recommended that buffaloes do not graze together with other animals including horse because they are able to harbour *T. evansi* in the body without showing clinical signs. It was also revealed by

Table 1. Distribution of Surra cases attacking horses and buffaloes in East Sumba District, Indonesia in 2010 - 2016

Periods	Year	Livestock									
		Horses					Buffaloes				
		Infected	Death	Population in risk	Morbidity (%)	Mortality (%)	Infected	Death	Population in risk	Morbidity (%)	Mortality (%)
I	2010	65	44	577	11.27	7.60	3	0	280	1.07	0.00
	2011	381	278	7921	4.81	3.51	48	30	1093	4.39	2.74
II	2012	350	350	7579	4.62	4.62	148	148	9602	1.54	1.54
	2013	47	16	9606	0.49	0.17	21	0	10132	0.21	0.00
	2014	34	5	5657	0.60	0.09	9	0	3894	0.23	0.00
	2015	7	0	2180	0.32	0.00	3	0	865	0.35	0.00
	2016	43	7	4041	1.06	0.17	12	0	1325	0.91	0.00

(Data source : Local livestock Agency Reports from 2010 – 2016)

Camoin et al. (2017) that all elephant infected by *T. evansi* in Thailand have been associated with the cattle and buffaloes.

Generally, morbidity of Surra attacking horses in East Sumba District in 2010 was greater than buffaloes for 11.27% and 1.07 %, respectively (Table 1). However, it seemed to be a similar rate for horses and buffaloes in 2011 for 4.81 and 4.39, respectively. In term of mortality, a number of horses killed by *T. evansi* infection was also relatively higher than buffaloes. According to Tehseen et al. (2017) and Camoin et al. (2017) that horses are more susceptible to *T. evansi* than buffaloes. They develop acute and most often fatal effect from the disease. The high mortality of horses in the Brazilian Pantanal was also reported by Seidl et al. (2001) reaching about 13% when there was no control program employed. In addition, horses are reported as an indispensable animal to the traditional extensive ranching management system employed like in East Sumba District (Seidl et al. 2001). Accordingly, the number of morbidity and mortality of horses caused by *T. evansi* infection were greater than other livestock.

During seven years (2010-2016), the highest mortality rate occurred in 2012, followed in 2011 and 2010. After 2012, the mortality rate declined dramatically and there was no animal death reported in 2015. However, in 2016, the morbidity of horses and buffaloes slightly increased. It was probably due to discontinues of prophylactic treatment. The local livestock agency in East Sumba District postponed treating livestock because they must wait for the drugs from the central government so that the treatment was temporary halted.

There are some various factors which contribute to the high mortality in East Sumba District. *Trypanosoma evansi* is able to develop a well-known strategy to escape from the host immune system by exhibiting various main membrane surface glycoproteins (Desquesnes et al. 2013; Holmes 2013). The hot and humid tropical climate in East Sumba District is suitable for the breeding of vectors (Jesse et al. 2016). The population density of vector plays a role in increasing the transmission of Surra from infected livestock to others (Menon & Mathew 2008).

Dongga (2013) also investigated factors influencing Surra widespread rapidly in East Sumba District. They were frequent livestock movement, infected and suspected livestock slaughtered without any prior inspection and traditional culture of Sumbanese (livestock used for ceremony of funeral and marriage). Another factor is the lack understanding on the knowledge of Surra control, transmission of the disease and how to treat the infected livestock. In addition, the farmers are reluctant to report the incidence of Surra when their livestock was infected by *T. evansi*.

To reduce Surra cases in East Sumba District, the local government conducted controlling and monitoring programs such as curative and prophylactic treatment, including vector control by spraying insecticides. In 2010 – 2011 (period 1), isometamedium was the only available drug in Indonesia and it was employed to treat livestock as curative and prophylactic in East Sumba District. The chemical is reported to able to cleavage trypanosomes kinetoplast Deoxyribonucleic acid-topoisomerase (kDNA–topoisomerase) complexes and cause disintegration of minicircle network within *T. evansi* kinetoplast through mechanisms which are independent of kDNA to eventually cause the parasite death (Kaminsky et al. 1997). In addition, Vreysen et al. (2013) and Desquesnes et al. (2013) stated that isometamedium (0.25-1.0 mg/kg BW) provides longer protection period. It will be up to 4 months at 1.0 mg/kg BW so that the drug is suitable for the purpose of prophylactic to treat herd in an endemic area. However, after implementing treatment strategy in period I (Figure 2), Surra cases remained a serious problem for the farmers and the disease widely spread to another sub-districts.

Garba et al. (2017) reported the effect of isometamedium chloride after being used to treat donkeys infected by *T. evansi*. The animals showed rapidly drop in mean parasitemia on day 2 (87.5%) and reach zero parasitemia on day 11 after treatment based on direct Micro Haematocrit Centrifugation Test (MHCT) and wet film. The parasite remained negative on days 21 and 50 according to Mice Inoculation Tests (MIC). However, the animals became positive parasitemia on days 100 indicating the presence of latent parasitemia status. Even though 60% of inoculated mice were positive of *T. evansi*, there was no mortality of mice recorded. The result demonstrated that isometamedium chloride did not completely remove the parasite from the treated animals, however, the remaining parasite remained rendered non-pathogenic and avirulent. Hutchinson et al. (2007) explained that the finding might be due to the resistance of *T. evansi* facilitated by switching off its surface glycoprotein coat. It seemed that the treatment strategy in period I in East Sumba District using isometamedium was ineffective or *T. evansi* has been resistant to the drug and infected other livestock. Unfortunately, due to a limited number of drugs and lack knowledge of Surra, the treatment was not followed by further observation. As a result, the morbidity and mortality of Surra in period I remained relatively high.

In period II, the treatment strategy was changed by using a combination of isometamedium and diminazene aceturate. The local vets in East Sumba District employed isometamedium as a preventive drug and diminazene aceturate as a curative drug (Delespaux et al. 2006; Wainwright 2009; Gutierrez et al. 2013). According to Radostits et al. (2006), diminazene aceturate is a chemocurative for Surra, the first line of treatment in trypanosomosis. This statement is in line with Miller (2003) mentioning that diminazene aceturate is stable, easy to use, and low toxicity so that it is relatively safe for the animals. The chemical was investigated to be highly bound to plasma proteins of buffalo calves and also bound to DNA and interferes with parasitic replication (Pandey et al. 2010; Melaku & Birasa 2013). In addition, diminazene aceturate has a strong affinity in the base pair AT, particularly in the minor groove region of DNA followed by inhibition of enzymes such as topoisomerase and nuclease (Gillingwater 2007; Kuriakose et al. 2012; Gutierrez et al. 2013).

Comparison of morbidity, mortality and fatality proportions of Surra attacking horses and buffaloes between period I and II in East Sumba District can be seen in Table 2. The result demonstrated that the proportion of morbidity and mortality for horses and buffaloes in period I was significantly greater than in period II ($P<0.0001$). Treatment strategy in 2012 - 2016 was able to decrease morbidity with proportion in horses and buffaloes for 1.44% and 0.66%, respectively. It indicated that treatment using a combination of isometamedium and diminazene aceturate (period II) might be more effective compared to using isometamedium alone (period I). The previous report demonstrated that diminazene aceturate and isometamedium are therapeutically effective against clinical Surra in buffaloes in India (Joshi & Singh 2000).

Diminazene aceturate is recommended only for therapeutic use because of rapidly excreted activity. The chemical was also able to binds to trypanosomal kinetoplast DNA and hinders the synthesis of RNA primers, generating an accumulation of replicating intermediates. As a result, the kDNA replication is inhibited (Sivajothi & Reddy 2016). However, trypanosomes have developed resistance if they were used regularly in low doses. Sivajothi & Reddy (2016) recorded that polypeptide profile of diminazene aceturate resistant isolates of *T. evansi* removed from buffaloes in India had different bands pattern from the previous finding indicating the presence of variations in the isolates. It could maintain the fatality case rate relatively high.

Based on the proportion of fatality analysis, the case fatality rate (CFT) of period II provided significantly higher than the period I ($P<0.0001$). Eloy & Lucheis (2009) mentioned that surra cases might

Table 2. Comparison of morbidity, mortality and fatality proportions of Surra attacking horses and buffaloes between period I and II in East Sumba District

Livestock	Criteria	Period I	Period II	P - value
Horses	Morbidity	5.25	1.44	< 0.0001
	Mortality	3.79	1.30	< 0.0001
	Fatality	70.20	90.21	< 0.0001
Buffaloes	Morbidity	3.71	0.66	< 0.0001
	Mortality	2.80	0.55	< 0.0001
	Fatality	58.82	82.94	0.0003

be decreasing but the severity of the disease could be worse. It indicated that some infected livestock would be difficult to cure. This might happen due to *T. evansi* in the filed more pathogen.

The impact of treatment on reducing the number of infected livestock and deaths on horses and buffalo based on treatment strategy period II was projected forward using forecasting analysis for the next 12 months by decomposition method. In this study, it was assumed that the main control of Surra using trypanocidals either as curative or preventive actions was better, because other controls such as vector control and livestock movement were assumed the same from period 1 and 2. Surra's projection of horses and buffaloes and the death of horses and buffaloes seemed to be on the downward trend in Figure 3 (horses) and Figure 4 (buffaloes). It indicated that the control program implemented in Sumba (a combination of isometamedium and diminazene aceturate) could reduce the incidence and of Surra and death of livestock by Surra in the next 12 months.

In term of mortality rate, the forecasting analysis showed that the number of horses killed by *T. evansi* infection in the next 12 months would be higher than buffaloes. The horse mortality would fluctuate ranged 0 to 49 cases however, the mortality rate in buffaloes was less ranged 0 – 8 cases. It was not surprised because the horse is more susceptible than buffaloes from Surra disease. Meanwhile, according to Coen et al. (2001) who applied Susceptible-Infected-Susceptible (SIS) model demonstrated that the buffaloes in Indonesia would be a clear infection in an estimated mean time period of 16.8 months (ranged 12.5 – 25.9 months) since the acquisition, either by drug treatment or self-cure.

Overall, the treatment strategy in East Sumba District by a combination of curative and preventive methods was relatively effective to deal with Surra. Seidl et al (2001) stated that the best method for treatment of *T. evansi* from a horse mortality perspective is the preventive strategy. The effectiveness of this strategy has been proven and employed in Africa, Bolivia and Paraguay. The preventive strategy would provide 100% protection for horses. In addition, the control of trypanosomosis in an endemic area like East Sumba District may ideally involve control of vectors, prophylactic treatment and good husbandry of the animal at risk. In addition, Jesse et al. (2016) mentioned that total elimination of trypanosomosis would be ineffective however, the treatment strategy to achieve tolerable level could be possible to deal with Surra. Due to East Sumba District has been an endemic area for Surra, the preventive treatment must be implemented regularly to anticipate the wider spread of the diseases and reduce the mortality of livestock, particularly in horses and buffaloes.

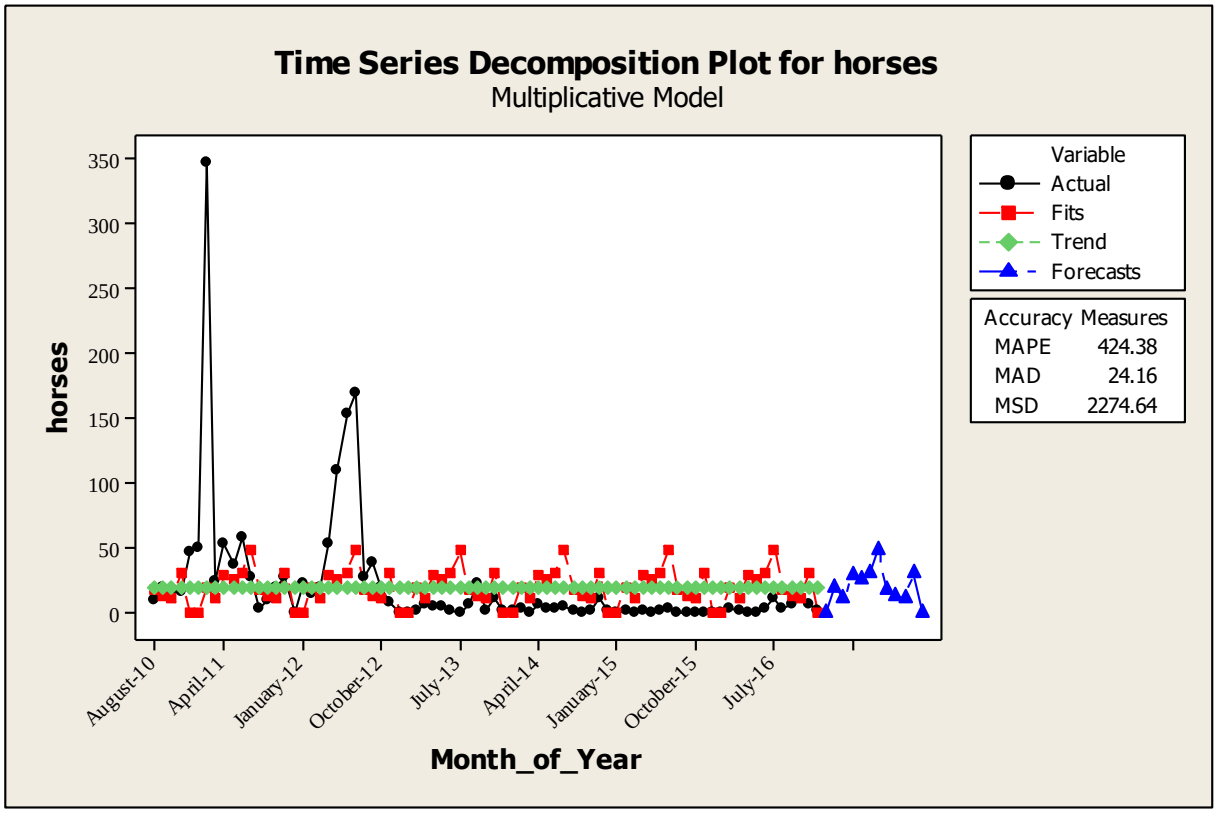


Figure 3. Sura cases projection on horses for the next 12 months based on forecasting study analysis.

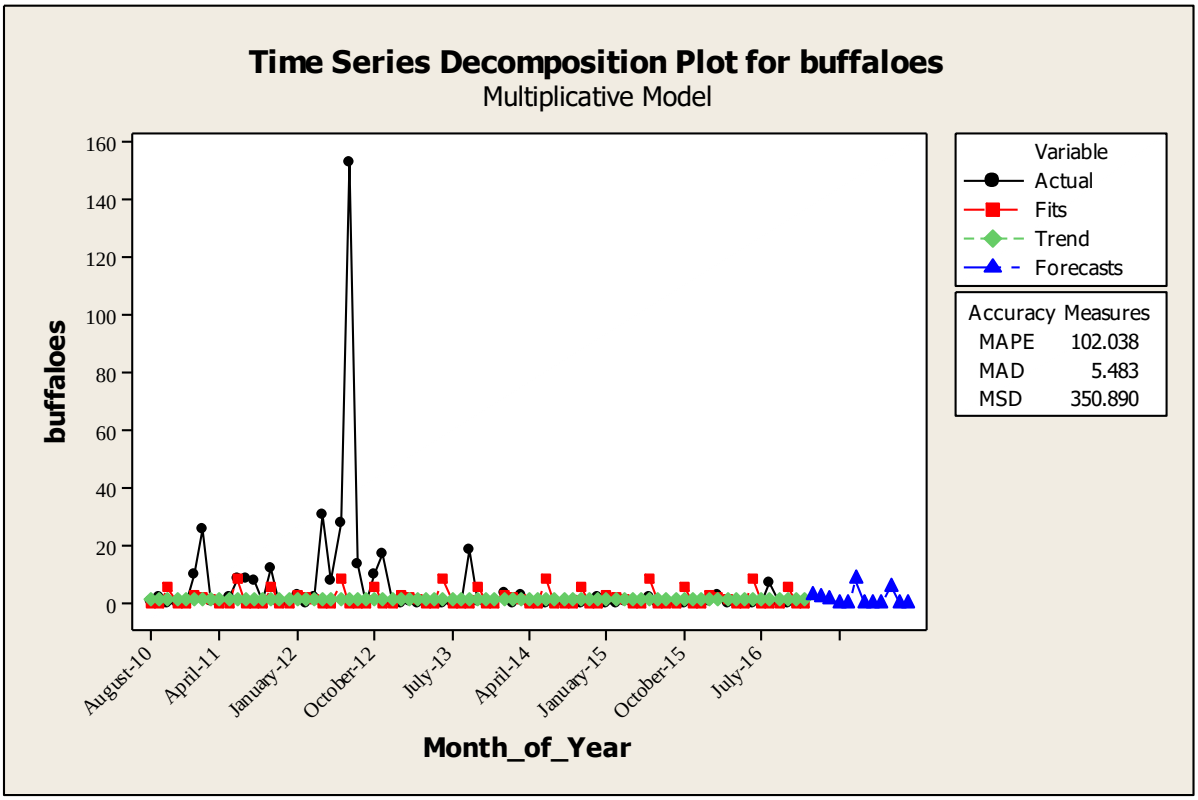


Figure 4. Surra cases projection on buffaloes for the next 12 months based on forecasting study analysis.

CONCLUSION

The strategy of Surra treatment by a combination of curative drug (isometamedium) and curative drug (diminazene aceturate) is able to significantly reduce the morbidity and mortality livestock in East Sumba District. According to the forecasting study, the Surra control program carried out continuously can reduce the case of Surra in East Sumba. The local livestock agency of East Sumba and the farmer should continue the program mainly using the combination of trypanocidal. The control of Surra is mainly based on the recognition of infested animal, so that the farmers or livestock keepers should observe the clinical signs and report to the local vet in order to obtain the proper treatment.

ACKNOWLEDGEMENTS

The author would like to thank drh. Martono, drh. Okto Rohi, drh. Rambu Peristiwati Pandjukang and the entire staff of the local livestock agency in East Sumba District for great cooperation during the study. The author also would express gratitude to Dr. Ir. Hamzah Bustomi MM for helping with statistical analyses. The study was supported by the Indonesia Agricultural Quarantine Agency - The Ministry Agriculture of the Republic of Indonesia. Rita Sari Dewi (RSD) and April H Wardhana (AHW) contributed equally in writing the manuscript.

REFERENCES

Aregawi WG, Agga GE, Abdi RD, Buscher P. 2019. Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. Parasites Vector. 12:1-125.

Camoin M, Kocher A, Chalermwong P, Yangtarra S, Thongtip N, Jittapalapong S, Desquesnes M. 2017. Adaptation and evaluation of an ELISA for *Trypanosoma evansi* infection (Surra) in elephants and its application to a serological survey in Thailand. Parasitology. 1-7.

Coen PG, Luckins AG, Davison HC, Woolhouse MEJ. 2001. Trypanosoma evansi in Indonesian buffaloes: evaluation of simple models of natural immunity to infection. Epidemiol Infect. 126:111-118.

Delespaux V, Chitanga S, Geysen D, Goethals A. 2006. SSCP analysis of the P2 purine transporter TcoAT1 gene of Trypanosoma congolense leads to a simple PCR-RFLP test allowing the rapid identification of diminazene resistant stocks. Acta Tropica. 100:96-102.

Desquesnes M, Dargantes A, Lai DH, Lun ZR, Holzmuller, Jittapalapong S. 2013. *Trypanosoma evansi* and Surra: A review and perspectives on transmission,

epidemiology and control, impact and zoonotic aspects. Biomed Res Inter. ID 321237:1-20.

Dongga RED. 2013. Adopsi Teknologi pengendalian surra oleh peternak kuda di Kabupaten Sumba Timur, Nusa Tenggara Timur (Thesis). [Bali (Indones)]: Universitas Udayana.

Eloy L, Lucheis S. 2009. Canine trypanosomiasis: aetiology of infection and implication for public health. J Venomous Anim Toxins Including Trop Dis. 15:589-611.

Garba UM, Sackey AKB, Lawal AI, Esievo KAN, Bisalla M, Sambo JS. 2017. Gross and histopathological alterations in experimental Trypanosoma evansi infection in donkeys and the effect of isometamedium chloride treatment. J Vet Sci Anim Husbandry. 5:1-10.

Gillingwater K, Buscher P, Brun R. 2007. Establishment of a panel of reference Trypanosoma evansi and Trypanosoma equiperdum strains for drug screening. Vet Parasitol. 148:114-121.

Gutierrez C, Gonzales-Martin M, Corbera JA, Tejedor-junco MT. 2013. Chemotherapeutic agents against pathogenic animal trypanosomes. In. Mendez –Vilas A, editor. Microbial pathogens and strategies for combating them: Science, technology and education. Madrid (Spain): Formatex Research Center. p. 1564-1573.

Harlan A, Setiawan BD, Marji. 2018. Peramalan jumlah kasus penyakit menggunakan jaringan saraf tiruan backpropagation (Studi kasus Puskesmas Rogotrunan Lumajang). J Pengembangan Teknologi Informasi Ilmu Komputer. 2:2781-2790.

Heizer J, Render B. 2009. Manajemen operasi. 9th ed. Jakarta (Indones): Salemba Empat.

Holmes P. 2013. Tsetse-transmitted trypanosomes their biology, disease impact and control. J Invertebrate Pathol. 112:11-14.

Hutchinson OC, Picozzi K, Jones NG, Mott H, Sharma R, Willburn SC, Caringgton M. 2007.Variant Surface Glycoprotein gene repertoires in Trypanosoma brucei have diverged to become strain-specific. BMC Genomic 8:234.

Jesse FFA, Bitrus AA, Abba Y, Sadiq MA, Hambali IU, Chung ELC, Ping FLP, Haron AB, Azmi M, Lila M, Saharee AA, Norsidin MJ. 2016. A clinical case of bovine trypanosomosis in an endemic farm in Malaysia. J Adv Vet Anim Res. 3:286-291.

Joshi SS, Singh B. 2000. Evaluation of therapeutic and chemo prophylactic efficacy of certain drugs against clinical surra in buffaloes. Indian Vet J. 77:895-897.

Kaminsky R, Schm id C, Lun ZR. 1997. Susceptibility of dyskinetoplastic Trypanosoma evansi and Trypanosoma equiperdum to isometamedium chloride. Parasitol Res. 83:816-818.

Kapa MMJ, Soemarno, Yanuwiyadi B, Suyadi. 2017. Sustainability status of biology dimension of local beef cattle development in the dryland region, Indonesia. J Econ Sustain Dev. 8:102-108.

Kundu K, Tewari AK, Kurup SP, Baidya S, Rao JR, Joshi P. 2013. Sero-surveillance for surra in cattle using native surface glycoprotein antigen from Trypanosoma evansi. Vet Parasitol. 196:258-264.

Kuriakose S, Mulene HM, Onyilagha C, Singh R, Jia P, Uzonna JE. 2012. Diminazeneaceturate (Berenil) modulates the host cellular and inflammatory response to Trypanosomacongolense infection. PLoS ONE. 7:1-8.

Luckins AG. 1998. Trypanosomiasis caused by Trypanosoma evansi in Indonesia. J Protozool Res. 8:144-152.

Local livestock Agency Report. 2010. Laporan Tahunan dan Peta Distribusi Penyakit Hewan Tahun 2010. Sumba Timur (Indones): Dinas Peternakan Kabupaten Sumba Timur.

Local livestock Agency Report. 2011. Laporan Tahunan dan Peta Distribusi Penyakit Hewan Tahun 2010. Sumba Timur (Indones): Dinas Peternakan Kabupaten Sumba Timur.

Local livestock Agency Report. 2012. Laporan Tahunan dan Peta Distribusi Penyakit Hewan Tahun 2010. Sumba Timur (Indones): Dinas Peternakan Kabupaten Sumba Timur. Indonesia.

Local livestock Agency Report. 2013. Laporan Tahunan dan Peta Distribusi Penyakit Hewan Tahun 2010. Sumba Timur (Indones): Dinas Peternakan Kabupaten Sumba Timur. Indonesia.

Local livestock Agency Report. 2014. Laporan Tahunan dan Peta Distribusi Penyakit Hewan Tahun 2010. Sumba Timur (Indones): Dinas Peternakan Kabupaten Sumba Timur. Indonesia.

Local livestock Agency Report. 2015. Laporan Tahunan dan Peta Distribusi Penyakit Hewan Tahun 2010. Sumba Timur (Indones): Dinas Peternakan Kabupaten Sumba Timur. Indonesia.

Local livestock Agency Report. 2016. Laporan Tahunan dan Peta Distribusi Penyakit Hewan Tahun 2010. Sumba Timur (Indones): Dinas Peternakan Kabupaten Sumba Timur. Indonesia.

Miller DB. 2003. The pharmacokinetics of Diminazene Aceturate and Intravenous Administration in the Healthy Dog (Thesis). [Pretoria (Indones)]: University of Pretoria.

Melaku A, Birasa B .2013. Drugs and drug resistance in African Animal Trypanosomosis: A Review. European J Bio Sci. 3:82-89.

Menon DG, Mathew L. 2008. Incidence of Trypanosoma evansi in Thrissur town. Vet World. 1:275-277.

Ndiha MRM, Apsari IAP, Dwinata IM. 2018. Prevalensi dan intensitas infeksi Trypanosoma evansi pada kuda di desa Kabaru, Kecamatan Rindi, Kabupaten East Sumba. Bul Vet Udayana. 1:70-75.

Nurulaini R, Premaalatha B, Zaini CM, Adnan M, Chandrawathani P, Fazly AZA, Enie AA, Ramlan M. 2013. Trypanosomiasis outbreak in deer, cattle, buffaloes and pigs in Perak. Malays J Vet Res. 4:55-58.

[OIE] Organization Internationale de Epizootic. 2012. *Trypanosoma evansi* infection. OIE Terrestial Manual. Chap.2.1.17. p. 1-4.

Pandey HK, Singh KK, Roy BK, Kumari S. 2010. Pharmacokinetics of Diminazene aceturate in buffalo calves. J Bioanalys Biomedic. 2:013-016.

Payne RC, Sukamto IP, Djauhari D, Partoutomo S, Jone TW, Luckin AJ, Boid R. 1991. *Trypanosoma evansi* infection in cattle, buffalo, and horses in Indonesia. Vet Pathol. 38:253-256.

Ponnudurai G, Sivaraman S, Rani N, Veeraoabduab C. 2015. An outbreak of Trypanomosis in buffaloes caused by diminazene resistant *Trypanosoma evansi*. Buffalo Bull. 34:1-4.

Putt SNH, Shaw APM, Woods AJ, Tyler L, James AD. 1988. Veterinary epidemiology and economic in Africa: A manual for use in the design and appraisal of livestock health policy. Berkshire (UK): University of Reading.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD. 2006. Veterinary Medicine: A textbook of disease of cattle, horses, sheep, pigs and goats. 10th ed. Elsevier Health Sciences. 2065 p.

Seidl AF, Moraes AS, Silva RAMS. 2001. *Trypanosoma evansi* control and horse mortality in the Brazilian Pantanal. Mem Inst Oswaldo Cruz, Rio de Janeiro. 96:599-602.

Sivajothi S, Chengalva V, Reddy BS. 2014. Detection of *Trypanosoma evansi* by different methods in bovines in Andhra Pradesh. J Adv Parasitol. 1:35-38.

Sivajothi S, Reddy BS. 2016. Polypeptide profiles of Diminazene Aceturate Resistant *Trypanosoma evansi* organisms isolated from a Buffalo. J Vet Sci Medic. 4:1-4.

Sumbria D, Singla LD, Kumar R, Bal MS, Kaur P. 2017. Comparative seroprevalence and risk factor analysis of *Trypanosoma evansi* infection in equines from different agro-climatic zones of Punjab (India). Revue Scientifique Technique International Office of Epizootics. 36:971-979.

Singh V, Singla LD. 2015. Trypanosomosis (Surra) in Livestock. Veterinary Parasitology. Department of Parasitology, College of Veterinary Science and Animal Husbandry, S.D Agricultural University. p. 330-305.

Tehseen S, Jahan N, Desquesnes M, Shahzad MI, Qamar MF. 2017. Field investigation of Trypanosoma evansi and comparative analysis of diagnostic tests in horses from Bahawalpur, Pakistan. Turk J Vet Anim Sci. 41:288-293.

The Regent of East Sumba issued Decree of Regent No: 185 / Disnak.524.3 / 570 / VII / 2010 and Instruction of Regent No: 147 of 2010 in July on the emergency response of threat of transmission of infectious animal diseases Surra in East Sumba district. 2010. Nusa Tenggara Timur-Indonesia

Vreysen MJB, Seck MT, Sall B, Bouyer J. 2013. Tsetse flies: their biology and control using area-wide integrated pest management approaches. J Invertebrate Pathol. 112:S15-S25.

Wainwright M. 2010. Dyes, trypanosomiasis and DNA: a historical and critical review. Biotech Histochem. 85:341-354.

AUTHOR GUIDELINES

Indonesian Journal of Animal and Veterinary Sciences, or IJAVS contains:

(i) Primary scientific manuscript of unpublished research results.
(ii) Elucidation of research methods and innovative techniques which is useful for research development.

AUTHOR GUIDANCE

Manuscript is written in good English, accompanied with abstract in English and Indonesian. Manuscript is typewritten on the A4 paper size with 2 spaces distance and 4 cm from left side, 3 cm from right side, 3 cm from top and bottom sides. We provide you with IJAVS Template that you can find in our website: http://medpub.litbang.pertanian.go.id/index.php/jitv.

SCRIPTWRITING SYSTEMATICS

1. **Title:**
   Should be comprehensive, but it is made as short as possible. Subtitle can be given if it needed.

2. **Name and Address of Author:**
   Author's name is written completely (without degree) and typewritten by CAPITAL letter. If the author is more than 1 person with different address, Arabic numbers superscript should be given behind each name. Author's address written under author's name, consisting of institution name and its complete address, made in line with number of index on behalf of the author and typewritten by ITALIC.

3. **Abstract:**
   Abstract is gift of manuscript, written in Indonesian or English, do not more than 250 words and stated in one paragraph. Abstract consists of backgound, purpose, material and methode, result and conclusion. The author's name (in CAPITAL form), publication year, manuscript title and journal name are listed before abstract content with layout as reference. Keywords are listed under the abstract, maximum 5 words.

4. **Introduction:**
   Is consisting of research background, issue, efforts which have been made, approach taken to solve the problem and research purpose.

5. **Materials and Methods:**
   Elucidating clearly about materials used and method carried out. If the material using animals in the experiment, please indicate that the animals are performed according to animal ethics and welfare. See ethical statement in the attachment.

6. **Results and Discussion:**
   It presents and discusses clearly and completely achieved research results based on the purpose. Result and discussion may be presented separately or united. Result description may be completed by consise tables and clear illustrations (black and white graphics, figures or photos) on separated page. Tabel description (on top) and illustrations (in bottom) should be clear and independent, so readers may easily understand the table without read the text. Discussion description consists of description of result and research mean and benefit associated with issue which will be solved. Measurement units both in table or illustrations use metric system.

7. **Conclusion:**
   It is a manuscript final summary.

8. **Acknowledgement:**
   It can be written if needed.

9. **References:**
   The author is recommended to use Mendeley Program (http://www.mendeley.com) and citation style of Taylor & Francis - Council of Science Editors (author-date). Mendeley program utilization is aimed to avoid mistakes in citations and references writting. Cited references (preferably, 80% is primary article and the last 10 years publication). and should not from unpublished articles such as practical guidance and research report, except thesis and dissertation. Download is allowed if it is from electronic magazine, genom database or patent.

   Citation in the references:
   Literatures in reference are written alphabetically based on the author's name. 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:**
Citation consists author's last name and publication year.

Example:

a. One author: ..... grow slower than lamb fed cattle's milk (Supriyati 2012). Supriyati (2012) formulates.....
b. Two authors: ..... expect, end maintenance weight (Khasrad & Rusdimansyah 2012). Khasrad & Rusdimansyah (2012) argued.....
c. Three authors or more: ..... based on DNA mitochondria analysis (Mtileni et al. 2011). Mtileni et al. (2011) reports.....
d. Same author cited from 2 different papers: (Purwadaria et al. 2003a, 2003b).
e. Author with same family name is written consecutive: (Dawson J 1986; Dawson M 1986).
f. Several different authors are written consecutive: (Kannan et al. 2000; Grandin 2007; Santosa et al. 2012).
g. Institution: CSA (2011).....

11. **Table:**
a. Standard word used is Times New Roman with 1 space distance and 11 of font size.
b. Title is simple, clear, and understable sentence without reads the manuscript.
c. Each column from table should has heading. Its unit separated from title by comma, in parentheses, or at its bottom.
d. Table description is written under the table with 1 space distance and 11 of font size. Data source is written under the table or in the table in own header.

Dividing line is made in form of horizontal.

12. **Figure and graphic:**
a. Title uses Times New Roman with 1 space distance and 11 of font size. It is a simple and clear sentence which is laid under the figure or graphic.
b. Line in graphic should show clearly difference of one and others, if there is more than one curve.
c. Clear contrast figure with proportionate size and high resolution to present the best performance.

Write figure or graphic source under the title.

1. If written manuscript is more than one, it needed an approval from the other authors by enclose initial behind each name.
2. Complete manuscript is sent in three copies to Editorial Board of IJAVS and its electronic file, or by online: http://medpub.litbang.pertanian.go.id/ index.php/jitv

The author is entitled to 1 original journal and 10 its reprints.

Jurnal Ilmu Ternak dan Veteriner

IJAVS Indonesian Journal of Animal and Veterinary Sciences

Center for Animal Research and Development
Indonesian Agency for Agricultural Research and Development
Padjajaran St. Kav. E59, Bogor 16128
Phone: 0251 - 8322185 | Fax: 0251 - 8380588
e-mail: jitvnak@yahoo.com/jitvnak@litbang.pertanian.go.id
http://medpub.litbang.pertanian.go.id/index.php/jitv/index

Dear
Editorial Board of Indonesian Journal of Animal and Veterinary Sciences
Indonesian Center for Animal Research and Development
Padjajaran St. Kav. E59, Bogor 16128

ETHICAL STATEMENT

Respect to paper submission to Indonesian Journal for Animal and Veterinary Science, by following this letter, I here:

Name :
Institution :
Title of Paper :

Acknowledging that the paper submitted is my own or team work, that:

- ☐ It is original or free from: a) fabrication; b) falsificasin; c) plagiarism; d) duplication; e) fragmentation; and f) data/content copyright infringement.
- ☐ It is obtained through **true** scientific meeting or free from: a) engineered scientific meeting; and b) not attended meeting.
- ☐ It is ensure the studies involving animals that are performed according to animal ethics and welfare.
- ☐ It is unpublished in other publications.

This acknowledgment is made honestly and responsible based on Regulation of Head of Indonesian Institute of Science Number 06/E/2013 about Code of Ethic of Researcher.

, 2019

Applicant,

_____

Author's colleague:

Name	Sign

Note:
Please sent statement letter with original signed and stamped **by post** to:
Technical Editor of Indonesian Journal of Animal and Veterinary Sciences
Pajajaran St. Kav. E59 Bogor 16128. Phone: (0251) 8322185 Fax. (0251) 8380588
Email: jitvnak@yahoo.com/jitvnak@litbang.pertanian.go.id
Website: http://medpub.litbang.pertanian.go.id/index.php/jitv/index

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Indonesian Center for Animal Research and Development
Indonesian Agency for Agricultural Research and Development
Padjajaran St. Kav. E59, Bogor 16128
Phone: 0251 - 8322185 | Fax: 0251 – 8380588
e-mail: jitvnak@yahoo.com/jitvnak@litbang.pertanian.go.id
http://medpub.litbang.pertanian.go.id/index.php/jitv/index

COPYRIGHT TRANSFER FORM

Title of Paper :

Author :

This paper is original and the author diverts its copyright to Indonesian Journal of Animal and Veterinary Sciences, incase if and when this paper is accepted.

Everyone listed as author in this paper had contributed to substation and intellectual and should be responsible to public. In case is notified a copyright infringement, it is responsible to the author, not responsible to Indonesian Journal of Animal and Veterinary Science.

This paper content is unpublished before and not being considered to be published in other journals.

, 2019

Approved by

Primary Author

Author's colleague:

Name	Sign

This form should be signed by **all authors and returned to the Editorial Board**. The form may be sent by post or email.

Acknowledgement

Editorial board and executive editor of Indonesian Journal for Animal and Veterinary Science (IJAVS) extent high appreciation to the expertises of peer reviewer of IJAVS (Volume 24 No. 1 2019).

1.	Prof. Dr. Arnold P. Sinurat	:	Feed and Nutrition - IRIAP
2.	Dr. Tatan Kostaman	:	Physiology and Reproduction - IRIAP
3.	Prof. Dr. Ni Wayan Kurniani Karja	:	Biotechnology Reproduction - Bogor Agricultural University
4.	Dr. Hartati	:	Animal Breeding and Genetics - Beef Cattle Research Station
5.	Dr. Elizabeth Wina	:	Animal Feed and Nutrition - IRIAP
6.	Prof. Dr. Endang Tri Margawati	:	Biotechnology - Research Center for Biotechnology, IIS
7.	Dr. Siti Darodjah Rasad	:	Animal Reproduction - Padjajaran University
8.	Ir. Tati Herawati, M.Agr.	:	Farming System - IRIAP
9.	Dr. Jakaria	:	Animal Breeding and Genetics - Bogor Agricultural University

We hope this good collaboration would be continued in the future in improving IJAVS quality.

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 24, Number 1, March 2019 ISSN 0853-7380 E-ISSN 2252-696X

LIST OF CONTENT

Registered in: