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PREFACE

In this edition, volume 24 No 4, we proudly present articles from animal and veterinary sciences including genetics; animal production and feed technology. The articles published in this edition are:
"Assessment of Genetic Relationships between Growth Traits and Milk Yield in Egyptian Buffaloes"; "The productivity of 4th Generation KUB-2 Chicken"; "Optimization of BS4 Enzyme Production with Different Substrate Thickness and Type of Trays"; "The Use of Coffee Husk as Napier Grass Substitution and Its Effect on Madura Cattle Performance"; "*Rhizopus oligosporus* Activity in Crude Extract and Powder Form to Reduce *Aspergillus flavus* and Aflatoxin Contamination in Corn"; and "Supplementation of Black Soldier Fly (*Hermetia illucens*) on Activity and Capacity Phagocytic Macrophage of Laying Hens".

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

Chief Editor

Bogor, December 2019

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LIST OF CONTENT

Assessment of Genetic Relationships between Growth Traits and Milk Yield in Egyptian Buffaloes

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(received 07-09-2019; revised 20-10-2019 ; accepted 31-10-2019)

ABSTRAK

Abu El-Naser IAM. 2019. Analisis kekerabatan genetik antara sifat-sifat pertumbuhan dan produksi susu Kerbau Mesir. JITV 24(4): 143-150. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2034

Data dalam penelitian ini diperoleh dari catatan bobot hidup dan produksi susu dari kerbau Mesir dalam status tiga laktasi pertama yang dipelihara di Balai Penelitian dan Produksi Ternak Mahallet Mousa dari sebanyak 987 catatan selama 16 tahun. Data tersebut dianalisa untuk menentukan estimasi parameter genetik menggunakan *animal model*. Nilai rataan (dalam kg) untuk BW; WW; W18; WFC; 1stMY; 2ndMY dan 3rdMY secara berturut-turut adalah 36,56; 96,95; 322,02; 462,09; 1561,53; 1755 dan 1837,71. Nilai heritabilitas *additive* langsung (h^2a) untuk sifat-sifat di atas secara berturut-turut adalah 0,31; 0,22; 0,24; 0,27; 0,23; 0,23 dan 0,17. Perhitungan yang tepat untuk heritabilitas *maternal* (h^2m) untuk sifat yang sama adalah 0,39; 0,34; 0,22; 0,40; 0,29; 0,31 dan 0,21. Nilai korelasi genetik antara semua sifat yang diamati adalah positif yang berkisar antara 0,02 hingga 0,55. Keakuratan nilai PBV's bervariasi mulai dari 62 hingga 76, 62 hingga 83 dan 41 hingga 77% untuk pejantan, induk dan anak secara berturut-turut. Nilai ini menunjukkan bahwa perbaikan genetik dapat dicapai dengan memanfaatkan nilai PBV's tersebut. Semakin timnggi nilai heritabilitas langsung dan maternal untuk BW dan WFC, semakin tinggi pula korelasi genetik antara produksi susu periode tiga laktasi pertama dan WW serta W18. Oleh karena itu, menjadi hal yang tepat untuk menseleksi anakan kerbau betina pada sifat bobot badan saat lahir dibandingkan bobot badan pada usia lainnya.

Kata Kunci: Nilai Pemuliaan, Kerbau Mesir, Parameter Genetik, Sifat-Sifat Pertumbuhan, Produksi Susu

ABSTRACT

Abu El-Naser IAM. 2019. Assessment of genetic relationships between growth traits and milk yield in Egyptian buffaloes. JITV 24(4): 143-150. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2034

Data in this study were collected from live body weight records and milk yield for the first three lactations of Egyptian buffaloes maintained at the Mahallet Mousa Experimental Station of Animal Production Research Institute, relying on 987 records of Egyptian buffaloes spread over 16 years. These data were analyzed to estimate genetic parameters using animal model. Overall means in kilograms of BW, WW, W18, WFC, 1stMY, 2ndMY and 3rdMY were 36.56, 96.95, 322.02, 462.09, 1561.53, 1755 and 1837.71, respectively. Direct additive heritability (h^2a) for mentioned traits were 0.31, 0.22, 0.24, 0.27, 0.23, 0.23 and 0.17, respectively. Corresponding computation of maternal heritability (h^2m) for same traits were 0.39, 0.34, 0.22, 0.40, 0.29, 0.31 and 0.21, respectively. Evaluation of genetic correlations among different all studied traits were positive and ranged from 0.07 to 0.83, while phenotypic correlations were positive and ranged from 0.02 to 0.55. Accuracy of (PBV's) varying from 62 to 76, 62 to 83 and 41 to 77% for sires, cows and dams, successively; pointing out the genetic improvement could be achieved through any pathway of them. Higher direct and maternal heritability for BW and WFC and genetic correlations between first three lactations milk yield and each of BW and WFC higher than genetic correlations between first three lactations milk yield and WW and W18. Therefore, it is appropriate to select buffalo female calves for live body weight at birth than for live body weights at other ages.

Key Words: Breeding Values, Egyptian Buffaloes, Genetic Parameters, Growth Traits, Milk Yield

INTRODUCTION

Buffalo is the more important dairy cattle in Egypt. Buffalo is adapted animal to the small-holder conditions and is raised under semi-extensive production systems. Therefore, it plays an important role in Egyptian agriculture. The Egyptian buffaloes are nearly to 3.9 million. Where contribution to milk production nearly 45.5% of total milk in Egypt (FAOSTAT 2013). The genetic parameters of growth efficiency traits will support in delicacy selection to improve the genetic ability of the breed for meat production in Murrah buffaloes (Thiruvenkadan et al. 2009). In Egyptian buffaloes Awad & Afify (2014) cleared that all growth traits from birth until weight at year and half age effectual for improvement through direct genetic

selection. In early period of rearing increased growth rate can decrease the expense of rearing the animal and thus raise greater economical to agriculturist. Environmental factors and maternal effects influencing birth weight and early growth rate of animals (Mandal et al. 2006). Estimate of heritability for weight at first calving was moderate so fortify essential genetic response through selection framework in Egyptian buffaloes (El-Bramony 2014). Birth weight and information on body weight at early ages in farm animal used in early selection criterion (Eyduran et al. 2009; Karakus et al. 2010). Akhtar et al. (2012) indicated that year and season of birth and weight of dam were significant ($P<0.05$) effect on birth weight, weaning weight and yearling weight in heifers of Nili-Ravi buffaloes. Genetic appraisement programs and culling or selection might used the weaning weight (Guidolin et al. 2012). In Holstein heifer, Van De Stroet et al. (2016) shown that milk yield in later life associated with pre weaning growth. And added that were not significantly associated with calf higher growth rates and future milk yield, while higher birth weight in lactating cows were associated with higher odds of survival to first lactation.

The aims of this present investigation were to estimate the genetic relationship between milk yield in first three lactations and weights at different ages from birth to first calving and breeding values for these traits in Egyptian buffaloes.

MATERIALS AND METHODS

Data collected from weight records and milk production in first three lactations of lactating Egyptian buffalo herds maintained at the Mahallet Mousa Experimental stations of Animal Production Research Institute (APRI), Ministry of Agriculture, using the records of 16 consecutive years from 2001 to 2016. The data comprised 987 lactation records of 395 dams and 113 sires. Traits considered in the study were birth weight (BW, kg), weaning weight (WW, kg), weight at eighteen months (W18, kg), weight at first calving (WFC, kg), first lactation milk yield (1stMY, kg), second lactation milk yield (2ndMY, kg) and third lactation milk yield (3rdMY, kg).

After birth, calves were sucking colostrum for the first three days of their life, and then, housed individually in calf pens bedded with rice straw until weaning (at fifteenth weeks of age). During this period calves were artificially suckling via natural milk, bring in pails depending on their weight. Moreover, bring calf starter at third week of their age up to the 15th weeks of rearing suckling, and berseem hay (*Trifolium alexandrinum*), water and mineral mixture were available freely to calves. After weaning, the animals involved in this study were kept under the same system of feeding and management in the stations. Lactating buffaloes were milked by hand or machine twice daily during the lactation period, and milk production was recorded daily. The animals were fed on Egyptian clover (*Trifolium Alexandrinum*) during (December to May) with concentrate mixture and rice straw. During (June to November), animals were fed on concentrate mixture, rice straw and limited amount of clover hay or silage. Buffaloes were feed according to their live weight, milk production and pregnancy status. Water is available for buffaloes at all times of the day in water troughs. Multi mineral licking blocks were available free for animals in the stalls. Buffaloes were inseminated during heat after 60 days postpartum, while heifers were inseminated when attained 350 kg of live body weight or 18-24 months of age.

Statistical analysis

Firstly, least squares means and analysis of variance of fixed effects on traits under investigation to calculate by using least squares analyses of variance by Mixed Model program of Harvey (1990).The following fixed model was used:

$$Y_{ijkl} = \mu + M_i + Y_j + F_k + e_{ijkl}$$

Where:

Y_{ijkl} = observation value
μ = overall mean
M_i = fixed effect of ith month of birth
Y_j = fixed effect of jth year of birth
F_k = fixed effect of kth farm, and
e_{ijkl} = random error term

Secondly, data were analyzed by animal model using multiple-trait derivative-free restricted maximum likelihood (MTDFREML) suite of programs (Boldman et al. 1995) to expectation the (co)variance components and genetic and phenotypic parameters for t studied traits. The subsequent model utilized:

$$Y = X\beta + Za + Mm + Wpe + e$$

Where:

Y = a vector of observations
β = a vector of fixed effects
a = a vector of additive genetic effects
m = a vector of maternal genetic effects
M = the incidence matrix relating records to maternal genetic effect
pe = a vector of environmental effects contributed by dams to records of their progeny (permanent environmental)

Table 1. Means, standard deviation (SD) and coefficient of variation (CV %) for studied traits in Egyptian buffaloes**.**

Traits	Mean	SD	CV%
WB	36.56	5.12	0.14
WW	96.95	15.52	0.16
W18	322.02	38.64	0.12
WFC	462.09	60.07	0.13
1stMY	1561.53	529.71	0.34
2ndMY	1755.00	577.11	0.33
3rdMY	1837.71	511.40	0.28

WB= birth weight, WW= weaning weight, W18=weight at eighteen months, WFC= weight at first calving, 1stMY=first lactation milk yield, 2ndMY=second lactation milk yield and 3rdMY= third lactation milk yield.

Table 2. Significance levels of some environmental factors affecting on studied traits under investigation.

Traits	F- Value and significance		
	Month of birth	Year of birth	Farm
WB	1.61ns	3.2**	17.76**
WW	1.46ns	5.57**	15.00**
W18	1.31nS	3.02**	4.73**
WFC	2.95**	4.54**	10.25**
1stMY	10.62**	21.23**	37.38**
2ndMY	8.16**	14.64**	53.15**
3rdMY	16.05**	16.32**	46.33**

**= significant at P<0.01, n.s= non significant, number of records =987, month of birth =12, year of birth = 16 and farm =3.

W = the incidence matrix relating records to permanent environmental effects

e = a vector of the residual effects. X and Z are incidence matrices relating records to fixed and genetic effects

Estimated breeding values via MTDFREML program for calculated best linear unbiased perdition (BLUP) of all animals' pedigree file for multi-traits analysis.

RESULTS AND DISCUSSION

Descriptive statistics

Means for WB, WW ,W18,WFC, 1stMY, 2ndMY and 3rdMY were 36.6, 96.95,322.02, 462.09, 1561.53, 1755 and 1837.71, kg, successively are presented in Table 1. The coefficient of variation for traits under study were varying from 0.12 to 0.34 %. Estimation of means for WW, WW, W18 in current study were slightly higher those reported by Awad & Afify (2014) in Egyptian buffaloes (36.30, 91.31 and 301.56 kg), successively. As well for birth weight in Murrah buffaloes estimated by Thiruvenkadan et al. (2009) (32.4 kg) and Salces et al. (2013) in water buffalo (35.10 kg). Likewise, the actual estimation of weight at first calving higher than (397.11) that obtained by El-Bramony (2014) in Egyptian buffaloes. The present means of 1stMY, 2ndMY and 3rdMY were higher than these 1175, 1552 and 1635 Kg found by El-Bramony (2011) in Egyptian buffaloes for 1stMY, 2ndMY and 3rdMY, respectively and 1347.2 kg for 1stMY estimated by Ahmad et al. (2013) in Nili-Ravi buffaloes.

While the actual mean for WFC was lower than reported by Yadav & Singh (2016) in Murrah buffaloes (503.73kg). In addition, 1stMY also was lower (1702.44 kg) than estimated by Yadav & Singh (2016) in Murrah buffaloes. Table (2) indicted that the effects of month and year of birth and farm had highly significant (P< 0.01) on studied traits except effect month of birth on BW, WW and W18 was not significant The present

results agree with obtained by Awad & Afify (2014) with relation to effect of farm on (BW, WW and W18) and influence of month of birth on WW. Contrariwise, for the affected WB, WW and W18 by month and year of birth. The current results agree with that obtained by El-Bramony (2011) for effect of farm on (1stMY), (2ndMY) and (3rdMY) and don't agree with present results for effect year of birth on mentioned traits.

Levels of birth weight

The present results indicated the highest milk yield in buffalo cows for first three lactations observed when level of birth weight were 36-40 kg for them, following by (41-45 kg), (46-50kg), (31-35 kg) and (25-30 kg). While the lowest milk yield of buffalo cows through three first lactations observed whereas, birth weight were >50 kg as in Table 4. It follows consider best birth weights for selection animals were ranged from 36-40 kg. Karakus et al. (2010) on Norduz kids shown that the life time yields in farm animals affected by birth weight characteristic raised in Bulgarian.

Genetic parameters

Heritability

Direct heritability (h^2_a) of WB, WW, W18, WFC, 1stMY, 2ndMY and 3rdMY were moderate, being 0.31, 0.22, 0.24, 0.27, 0.23, 0.23 and 0.17, respectively.

Estimation of h^2_a for BW in the current investigation was lower than those obtained by Fooda (2005) (0.40) for Egyptian buffaloes, Gupta et al. (2015) (0.349) in Murrah buffaloes and Awad & Afify (2014) for Egyptian buffaloes (0.32). While was higher than estimated by Kaygisiz et al. (2012) for BW (0.15) on Holstein Friesian. The current h^2_a estimate for WW was lower that calculated by Awad & Afify (2014) for Egyptian buffaloes being 0.40. In addition, h^2 estimate for W18 lower than, those perceived by Gupta et al. (2015) Murrah buffaloes (0.252) and Agudelo-Gómez et al. (2015) Colombia buffaloes (0.44). The present value of h^2 for WFC was higher than that estimated by Yadav & Singh (2016) in Murrah Buffaloes (0.08). The actual h^2 for 1stMY, 2ndMY and3rdMY in (Table 4) were higher than noticed by El-Bramony (2011) in Egyptian buffaloes for these traits (0.22, 0.16, 0.13), respectively and Yadav & Singh (2016) for 1stMY in Murrah Buffaloes (0.22) but was lower than that obtained by Gupta et al. (2015) for 1st MY (0.243) in Murrah buffaloes. The h^2_m for WB, WW, W18, WFC, 1stMY, 2ndMY and 3rdMY were moderate (0.39, 0.34, 0.22, 0.40, 0.29, 0.31 and 0.21), respectively. The present result for BW was lower than that stated by Kaygisiz et al. (2012) Holstein Friesian (0.56), while the current estimate of h^2_m for BW, WW and W18 were higher than those reported by Awad & Afify (2014) in Egyptian buffaloes 0.38, 0.26 and 0.09, respectively.

Correlations

Estimates of r_{am} in traits under current study were negative and varying from (-0.02 to-0.01) are presented in Table 4. The actual results agreement with those stated by Falleiro et al. (2013) in Mediterranean buffaloes, Awad & Afify (2014) in Egyptian buffaloes and Chud et al. (2014) in Nellore beef cattle. The estimation of genetic correlations (r_g) among body weight at different ages from birth to first calving ranged between 0.07 and 0.49. The highest value found between BW and WFC while the lowest between BW and W18. The present r_g between BW and WW was

Table 3. Effect of different birth weight levels of female calves on first, second, third lactations milk yield in Egyptian buffaloes.

Level of birth weights	N	Traits		
		1stMY	2ndMY	3rdMY
		Mean ± SE	Mean ± SE	Mean ± SE
25-30 kg	83	1367.37±62.62	1515.5 ±43.94	1631.38 ±60.42
31-35 kg	89	1447.88±55.73	1730.5±67.10	1697.00 ±73.92
36- 40 kg	425	1783.04±45.78	1946.32 ±75.99	2029.35 ±54.88
41-45 kg	324	1553.00±59.07	1935.53 ±60.75	1948.56 ±72.80
46-50 kg	48	1465.23±85.13	1759.73 ±53.81	1698.95 ±42.96
>50 kg	18	1243.76±76.541	1481.03 ±40.37	1584.46 ±43.75

1stMY=first lactation milk yield, 2ndMY=second lactation milk yield and 3rdMY= third lactation milk yield

Table 4. Estimation of direct and maternal heritability and direct maternal genetic correlation for studied traits.

Estimate	Traits						
	WB	WW	W18	WFC	1stMY	2ndMY	3rdMY
h^2_a	0.31	0.22	0.24	0.27	0.23	0.23	0.17
h^2_m	0.39	0.34	0.22	0.40	0.29	0.31	0.21
r_{am}	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.02
c^2	0.23	0.24	0.35	0.20	0.15	0.17	0.22
e^2	0.07	0.20	0.19	0.13	0.33	0.29	0.40

h^2_a= direct heritability, h^2_m= maternal heritability, r_{am}= direct maternal genetic correlation, c^2 = fraction phenotypic variance to permanent environmental and e^2= fraction phenotypic variance due to residual effects.

Table 5. Correlations among studied traits in present investigation in Egyptian buffaloes**.**

Trait	Correlations					
	$Trait_2$	r_{a1a2}	r_{p1p2}	r_{e1e2}	r_{pe1pe2}	r_{m1m2}
WB	WW	0.15	0.45	0.51	0.27	0.30
	W18	0.07	0.09	0.21	-0.14	0.23
	WFC	0.49	0.33	-0.06	-0.48	0.54
	1stMY	0.58	0.28	0.03	0.28	0.11
	2ndMY	0.33	0.25	-0.53	0.43	0.18
	3rdMY	0.45	0.38	-0.03	0.18	0.28
WW	W18	0.12	0.11	0.04	-0.14	-0.26
	WFC	0.40	0.14	0.66	-0.67	0.10
	1stMY	0.22	0.24	-0.47	0.31	0.18
	2ndMY	0.25	0.12	-0.14	0.09	0.10
	3rdMY	0.26	0.22	0.08	-0.07	0.32
W18	WFC	0.41	0.14	-0.13	0.12	0.08
	1stMY	0.11	0.02	0.02	-0.013	-0.06
	2ndMY	0.18	0.04	-0.16	-0.08	0.01
	3rdMY	0.15	0.03	0.08	0.04	-0.14
WFC	1stMY	0.50	0.23	-0.26	0.39	0.07
	2ndMY	0.38	0.26	0.01	0.28	0.15
	3rdMY	0.42	0.29	0.32	0.19	0.15
1stMY	2ndMY	0.63	0.55	0.26	0.37	0.23
	3rdMY	0.82	0.23	0.19	0.03	0.45
2ndMY	3rdMY	0.83	0.37	0.18	0.13	0.23

r_{a1a2} = genetic correlation between trait1, 2 and so on, r_{p1p2} = phenotypic correlation between traits 1, 2 and so on, r_{e1e2} = residual environmental ratio between traits 1, 2 and so on and r_{pe1pe2} = permanent environmental ratio between traits 1, 2 and so on r_{m1m2}= maternal genetic correlation between traits1, 2.

Table 6. Expected of breeding values for sires, cows and dams and accuracies %, for studied traits in Egyptian buffaloes.

Traits	Breeding Values			
	Minimum ± SE	Maximum ± SE	Accuracy, %	Range
Buffalo sires (EBV's)				
BW, kg	-2.95±0.97	2.90±0.89	62 – 76	5.85
WW, kg	-11.14±1.50	8.77±1.54	68 – 76	19.91
W18, kg	-38.81±1.59	44.13±1.65	70 – 73	81.94
WFC, kg	-63.14±1.55	74.18±1.52	73 – 75	137.27
1stMY, kg	-254.41±1.30	279.05±1.33	73 – 75	533.46
2ndMY, kg	-377.50±1.46	346.50±1.47	65 – 66	724.00
3rdMY Y, kg	-260.06±1.24	229.49±1.61	64 – 72	559.52
Buffalo cows (EBV's)				
BW, kg	-4.42±0.71	4.74±0.77	71-.72	9.16
WW, kg	-15.11±1.36	15.10±1.35	76 – 78	30.21
W18, kg	-40.19±1.34	53.32±1.21	81- 83	93.51
WFC, kg	-60.5±1.81	63.03±1.81	67 -73	123.53
1stMY, kg	-227.72±1.73	349.62 ±1.52	62 –70	577.34
2ndMY, kg	-336.81±1.64	300.92±1.60	66 – 77	637.37
3rdMY, kg	-251.22±1.24	456.93±1.24	69 – 71	708.15
Buffalo dams (EBV's)				
BW, kg	-2.61±0.95	3.24±0.95	47 - 48	5.85
WW, kg	-12.10±1.57	8.25±1.57	41 - 51	20.35
W18, kg	-44.76±2.06	54.32 ±2.08	43 - 45	99.08
WFC, kg	-64.48±1.53	63.74±1.52	73 - 74	128.22
1stMY, kg	-290.26±1.35	410.30±1.33	73 - 74	700.56
2ndMY, kg	-363.66±1.44	378.26±1.46	66 - 69	741.92
3rdMY, kg	-356.14±1.28	430.08±1.11	67- 77	786.22

WB= birth weight, WW= weaning weight, W18=weight at eighteen months, WFC= weight at first calving, 1stMY=first lactation milk yield, 2ndMY= second lactation milk yield and 3rdMY =third lactation milk yield.

nearest to that obtained by Chud et al. (2014) in Nell Nellore beef cattle (0.14) and lower than that obtained.

Agudelo-Gómez et al. (2015) noticed that, r_g between WW and W18 being positive and high (0.72) in Colombia buffaloes and Gupta et al. (2015) clarified that r_g between BW and 1stMY was 0.30. In contrary El-Bramony (2014) obtained lower and negative correlation between 1stMY and WFC (-0.22). The phenotypic correlations (r_p) among WB, WW, W18, and WFC ranged from 045 to 0.09 and from 0.37 to 0.55 among 1stMY, 2ndMY and 3rdMY as in Table 5. In Egyptian buffaloes, El-Bramony (2011) estimated the phenotypic correlations among 1stMY, 2ndMY and 3rdMY ranging 0.23 to 0.44. Awad & Afify (2014) estimated the phenotypic correlations among BW, WW and W18 as from 0.44 to 0.71. The maternal correlations (r_m) among traits were positive except the correlation between W18 and each of WW, 1stMY and 3rdMY. in Table 5. Awad & Afify (2014) obtained the r_m among WB, WW and W18 were ranged from (0.45 to 0.91) in Egyptian buffaloes. Genetic correlations as in table (5) showed that r_g between BW and WW was smaller (0.15), indicating that postnatal growth performances can be improved without increasing birth weight. El-Awady et al. (2005) came to the same correlation. They stated that the r_g between BW and WW was smaller than 50% and was between BW and daily gain negative that indicted the postnatal growth can be increased without increasing BW. Additionally the present results between growth traits and milk yield

in first three lactations were taken the previous same trend.

Breeding values

Breeding values consider best measurement able to discern the genotype best animals and it's bring about accurately selection. Accuracy of breeding values, varying from 0.62 to 76, 62 to 83 and from 41 to 77% for sires, cows and dams, in succession. As a result genetic improvement could be realized through whoever sires or cows or dams. The sires breeding values (Kg) for WB, WW, W18, WFC, 1stMY, 2ndMY and 3rdMY ranged from -2.95 to 2.90, -11.14 to 8.77, -38.81 to 44.13, -63.14 to 74.18 and -254.41 to 279.05, -377.50 to 346.50, and -260.06 to 229.49, respectively. Corresponding value (Kg) for cows ranged between -4.42 to 4.74, -15.11 to 15.10, -40.19 to 53.32, -60.5 to 63.03, -227.72 to 349.62, -336.81 to 300.92 and -251.22 to 456.93 kg, for the same above traits, consecutively. In this facet, breeding values (Kg) for the aforementioned traits for dams were -2.61 to 3.24, -12.10 to 8.25, -44.76 to 54.32, -64.48 to 63.74, -290.26 to 410.30, -363.66 to 378.26 and -356.14 to 430.08, respectively in Table 6. Sanghuayphrai et al. (2013) cleared that the breeding values use as base to genetic selection, although high accurate EBV appropriate phenotypic data for weaning weight of swamp buffaloes.

El-Awady et al. (2005) showed that the range of breeding values for BW and WW were 4.9 and 22, 8.19 and, 26 and 5 and 26 kg, for sire, cow and dam respectively, and noticed that the cows breeding values having high accuracy (over 80%) in Egyptian buffalo calves.

CONCLUSION

The current results indicated that the influence of direct and maternal heritability for BW and WFC were high efficiency. Additionally, a moderate genetic correlations between each of BW and WFC and milk yield in the first three lactations and higher than relations between each of WW and W18 and those lactations milk yield. It is indicating that female calves can be selected to milk production from birth based as birth weight, which will lead to buffalo cows with longer productive lives and higher profitability.

Moreover, higher ranges and accuracies of estimated breeding values through any pathway of sires or cows or dams cleared that increased genetic divergence among individuals are founding, therewith genetic improvement could be achieved.

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The productivity of 4th Generation KUB-2 Chicken

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ABSTRAK

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Ayam KUB-2 adalah ayam hasil seleksi dari KUB-1 untuk produksi telur dan warna *shank* kuning. Ayam KUB-1 sebanyak 64% mempunyai warna bulu dengan warna dasar hitam. Warna bulu hitam berpengaruh terhadap warna kulit karkas yang agak gelap, sehingga kurang disukai konsumen. Warna *shank* kuning mempunyai korelasi positif dengan warna kulit karkas yang cerah berwarna kuning. Dalam penelitian ini digunakan sebanyak 517 ekor ayam dara KUB-2 hasil seleksi generasi ke-4 yang terdiri atas 194 ekor KUB-2_{kk} (kaki kuning) dan 323 ekor KUB-2_{nk}(non kuning). Semua ayam ditempatkan pada kandang individu dan dievaluasi selama 6 bulan pertama masa produksi telur. Peubah yang diamati adalah umur pertama bertelur (UPB), bobot induk pertama bertelur (BIPB), bobot telur pertama (BTP), rataan bobot telur (RBT), produksi telur (PT) selama 6 bulan pertama (24 minggu), mortalitas, konsumsi dan konversi pakan. Hasil penelitian menunjukkan bahwa UPB, BIPB, BTP dan PT ayam KUB-2_{kk} dan KUB-2_{nk} generasi ke-4 tidak berbeda nyata (P>0,05). Pada KUB-2_{kk} dan KUB-2_{nk} menunjukkan masing-masing untuk UPB: 156,2 hari dan 158,1 hari; BIPB: 1788 g dan 1808 g, dan BTP: 31,32 g dan 31,34 g. PT selama 24 minggu masing-masing untuk KUB-2_{kk} dan KUB-2_{nk} generasi ke-4 adalah 103,3 butir atau 61,5% dan 101,9 butir atau 60,7%, dan FCR 25-43 minggu masing-masing untuk KUB-2_{kk} dan KUB-2_{nk} generasi ke-4 adalah 3,53 dan 3,54. Rataan bobot telur meningkat seiring meningkatnya umur ayam, mortalitas untuk seluruh populasi hanya 0,98%.

Kata Kunci: Ayam KUB-2 Generasi Ke-4, Produksi Telur, Kaki Kuning

ABSTRACT

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KUB-2 line of chicken has improved local chicken selected from the KUB-1 chicken line. KUB-2 was selected for more egg production and yellow shank. KUB-1 chicken has 64% various of black feather color, which sometimes tends to have unpreferred dark carcass. Yellow shank color has a positive correlation with the skin color of carcass. As many as 517 pullets of KUB-2 at 4th generation were divided into two groups of 194 pullets of KUB-2_{kk} (yellow shank) and 323 pullets of KUB-2_{nk} non-yellow shank). The chickens were raised intensively in the individual cages for the 24 weeks observation. Variables measured were age at first egg (AFE) bodyweight at first egg (BWFE), egg weight at first egg (EWFE), average egg weight (AEW), average egg production (AEP) during 24 weeks, feed conversion ratio (FCR) of 25-43 weeks of age, and mortality. The result showed that there was no statistically significant different (p>0.05) between KUB-2_{nk} and KUB-2_{kk} respectively for AFE of 156.2 d and 158.1 d, for BWFE of 1788 g and 1808 g, for EWFE of 31.32 g and 31.34 g, for AEP_{24} of 103.3 eggs or 61.5% and 101.9 eggs or 60.7%, and for $FCR_{25\text{-}43}$ of 3.53 and 3.54. AEW increased with increasing age of hen, the mortality of the whole population was 0.98%.

Key Words: 4th Generation KUB-2 Chicken, Egg Production, Yellow Shank

INTRODUCTION

KUB-2 line of chicken has been selected from its ancestor of the KUB-1 line. The KUB-1 is a moderate egg type of local chicken invented by the Indonesian Research Institute for Animal Production (IRIAP) and was released for the commercial by the Agricultural Minister No. 274/Kpts/SR.120/2/2014 (Direktorat Jenderal Peternakan dan Kesehatan Hewan 2014). The production of KUB-1 has been licensed to a few national medium and small local chicken breeders since early 2017 (IRIAP, 2018 Pers Com). The KUB-1 produces egg up to 50% henday production (HDP) or about 180 eggs/hen/year. Sartika et al. (2013) recognized the feather color of the population of KUB-1 was 64% dark-color base with shank color of about 74% dark. Shank color has a positive correlation with carcass skin and beak color (Gao et al. 2017). Chinese local chickens, Xianju and Baler Yellow chickens have yellow shank, beak, with feather color of red-yellow

buff is selected for egg type chicken line and China Chongren Patridge and Dongxiang Blue which have black and grey shank colors, usually have white or grey skin, beak with feather color of black-greyish and reddish in male and black spotted in female, is bred for egg and meat (Gao et al. 2017). In fact, Indonesia has chicken breeds that have yellow shank and skin with a yellow-reddish buff feather with Columbian pattern. The breeds called Merawang and Nunukan, which were originally brought from China a hundred years ago (Sartika et al. 2016). Genetically the yellow shank is influenced by the beta-carotene dioxygenase 2 (BCDO2) gene, which is used as a genetic marker for identifying the gene that determines shank color (Eriksson et al. 2008; Gao et al. 2017). They furthermore reported that yellow shank color is generated from or belongs to the cluster of grey jungle fowl (*Gallus sonneratii*) whilst white/grey/black are generated from or belong to a cluster of red jungle fowl (*Gallus gallus*).

KUB-2 line was obtained by selecting KUB-1 as it was still a high variation in egg production. In another word, the KUB-2 line was not the product of crossbreeding the KUB-1 line with other local breeds. The improvement of the breed through selection is time-consuming which is resulting in more permanent product quality (Padhi 2016), whilst crossbreeding may increase productivity quickly, but it will deplete its original genetic resources very fast (Dessie et al. 2011). Some developing countries like Ethiopia (Mengesha 2012; Hailu et al. 2014; Gebremariam et al. 2017; Terfa et al. 2019), Kenya (Magothe et al. 2015; Kamau et al. 2019), Nigeria (Nwogwugwu et al. 2018), Ghana (Osei-Amponsah et al. 2015), India (Haunshi et al. 2019), and Thailand (Suphawadee & Tuan 2016), have increased their local breeds of chicken either through selection or crossbreeding. Eventually in Indonesia, the work of improvement of local chicken breeds has been initiated as well (Sartika et al. 2013; Iskandar & Sartika 2014).

The aim of the research was to explore the development of laying performance of the 4th generation of KUB-2_{kk} (yellow shank) and KUB-2_{nk} (non-yellow shank), selected for more egg. The aim was also to see whether the selection for yellow shank would influence laying performance of the 4th generation of KUB-2 line of chicken.

MATERIALS AND METHODS

The KUB-2 line was selected from the KUB-1 line for egg production and yellow shank. The 40% of the highest egg production of each generation of KUB-2 line was selected and generated KUB-2_{kk} line of chicken having a yellow shank and KUB-2_{nk} line of chicken having no yellow shank. Five hundred and seventeen of 4th generation KUB-2 pullets age of 16 weeks, were grouped into sublines of 194 KUB-2_{kk} and 323 KUB-2_{nk} pullets.

The pullets were confined in the individually wire-cage of 35 cm height x 40 cm width x 40 cm length. Feed composed of 75% commercial layer feed (17% protein, 2850 kcal ME/kg, 3.4% Ca, 5% fiber) mixed with 24% wheat pollard and 1% mineral premix (Sinurat et al. 2014, with modification changes rice bran to wheat pollard). The feed contained 16.1% crude protein, 2800 kcal ME/kg, 3.2% Ca, 0.5% total P, 0.9% lysine, 0,45% methionine, and 6.15% crude fiber, was daily served at the amount of 100 g/pullet. Drinking water was served ad libitum. Feed supplement egg stimulant was added to drinking water as much as 50 g per 100 liters water given in five days continuously, especially in the extreme climate, such as heavy rain or dry and moist environment. In addition, the husbandry of the experiment pullets was fulfilled animal welfare condition under the regulation of the Indonesian Agency for Agricultural Research and Development (IAARD) Animal Welfare Commission of IRIAP/A/01/2018.

Variables measured were an age at first egg (AFE), bodyweight at first egg (BWFE), egg weight at first egg (EWFE), average egg weight (AEW), average egg production of 20-43 weeks of age (AEP_{24}), feed conversion ratio (FCR) and mortality. The data were then analyzed by t-test using software Minitab version 14.

RESULTS AND DISCUSSION

Age at first egg (AFE), body weight at first egg (BWFE), and egg weight at first egg (EWFE)

Age at first egg (AFE), bodyweight at first egg (BWFE), and egg weight at first egg (EWFE) of KUB-2 chicken at 4th generation of selection are presented in Table 1. AFE of KUB-2_{kk} (158.1 ± 26.2 days or 22.6 weeks) was not significantly ($p>0.05$) different from AFE of KUB-2_{nk} (156.2 ± 21 days or 22.3 weeks). The average AFE of whole populations was 156.9 days or around 22.4 weeks, which was ideal as it was shorter compared to their parent of KUB-1 line, ranging from 166.9-183.1 days (23.8 – 26.2 weeks) (Sartika et al. 2013). Acceleration of AFE after eight generations of selection was also reported by (Haunshi et al. 2019) in local Aseel Indian chicken from 159.7 days (22.8 weeks) to 173.9 days (24.8 weeks). (Wondmeneh et al. 2016) reported that Ethiopian local Horro chicken responded AFE at 159.5 days (22.8 weeks) to seven generations of selection. They also fund out the quickest first lay was at 112 days (16 weeks) of age for few hens, which was increasing slowly up to 19 weeks of age. At the age of 140 days (20 weeks), there was only 10% of the population laid an egg, which was adopted as the first date of proper egg production recording.

Table 1. Age at first egg (AFE), bodyweight at first egg (BWFE), and egg weight at first egg (EWFE) of KUB-2 chicken at 4th generations of selection

	No (pullets)	Age at first egg (AFE)					
		Average (days)	St.deviation (days)	Min (days)	Maks (days)	t-value	P-value
KUB-2_{kk}[1)]	194	158.1	26.2	112	245	0.90	0.367
KUB-2_{nk}	323	156.2	21.0	114	244		
		Bodyweight at first egg (BWFE)					
		(g)	(g)	(g)	(g)		
KUB-2_{kk}	194	1808	240	1220	2782	0.92	0.356
KUB-2_{nk}	323	1788	231	1178	2649		
		Egg weight at first egg (EWFE)					
		(g)	(g)	(g)	(g)		
KUB-2_{kk}	194	31.34	5.44	19	52	0.27	0.790
KUB-2_{nk}	323	31.32	4.67	17	51		

[1)] kk = Yellow shank; nk = Non-yellow shank

Table 2. Egg weight of KUB-2 chicken at 4th generation of selection

Age at week		KUB-2_{kk}[1)] (n=190)	KUB-2_{nk} (n = 322)
18-20	Average (g)	30.01±2.41	29.99±2.10
	CV (%)	8.04	7.03
21-24	Average (g)	35.70±1.56	35.71±1.88
	CV (%)	4.36	5.27
25-28	Average (g)	39.10±1.05	39.16±1.06
	CV (%)	2.57	2.70
29-32	Average (g)	41.68±1.04	41.60±0.73
	CV (%)	2.49	1.75
33-36	Average (g)	43.50±0.88	43.69±0.56
	CV (%)	2.04	1.28
37-40	Average (g)	44.51±0.30	44.79 ± 0.75
	CV (%)	0.66	1.68
41-44	Average (g)	45.65±0.48	46.09±0.42
	CV (%)	1.04	0.92

[1)] kk = Yellow shank; nk = Non-yellow shank

Table 3. Egg production of KUB-2 chicken at 4th generation during the first 24 weeks

	N	Average		St.deviation		Minimum		Maksimum		Values	
	(pullets)	(eggs)	(%)	(eggs)	(%)	(eggs)	(%)	(eggs)	(%)	(t)	(P)
KUB-2_{kk}[1)]	190	101.9	60.7	27.8	16.5	5	2.95	154	91.7	-0.57	0.57
KUB-2_{nk}	322	103.3	61.5	25.9	15.4	5	2.98	151	89.9		

[1)] kk = Yellow shank; nk = Non-yellow shank

Assefa et al. (2018) reported that Ethiopian local chicken, which was raised in high altitudes had longer AFE than when it was raised in lower altitudes. They also reported that AFE had a negative correlation with egg production ($p<0.01$; R= -0.57), which meant the faster the AFE the lower egg production. Mengesha (2012) reported also on Ethiopian local chicken, which had AFE 157-161 days or about 22.7 weeks of age at intensive husbandry, while under semi-intensive, their AFE was longer (25 – 25.7 weeks). So Shumuye et al. (2018) reported on Koekoek Ethiopian local chicken which had AFE of 6 months when raised in the villages. Further Shumuye et al. (2018) added their information on Ethiopian indigenous, Sasso and Koekoek raised in high altitude showed their AFE of 252, 162 and 184 days with egg production of 53.7, 137 and 148 eggs/hen/year, respectively. When those breeds were raised in low altitude their AFE was quicker (224, 147 and 148 days), but egg production was also lower (44.6, 129 and 115 eggs/hen/year respectively for Ethiopian indigenous, Sasso and Koekoek).

Bodyweight of the hen at the first egg laid (BWFE) of KUB-2_{kk} (1808 ± 240 g) and of KUB-2_{nk} (1788 ± 231 g) were not significantly different ($p>0.05$). The figures are higher than their parents, KUB-1 (1600 g/hen) (Sartika et al. 2013; Iskandar & Sartika 2014). BWFE of 1600 -1800 g at the age of 22 weeks seems to the ideal weight for a hen to start producing the egg. Matawork et al. (2019) found out the BWFE of 24 weeks pullet of Ethiopian indigenous breed across agroecosystem of 1480 g, had lower egg production than the KUB-2 line. However, Haunshi et al. (2019) reported that Indian local Aseel selected for 5 generations had BWFE of 1669±10.6 g, which is to some extent close to the result of KUB-2 of this experiment.

Egg weight at first egg (EWFE) of KUB-2_{kk} (31.34 ± 5.44 g) and KUB-2_{nk} (31.32 ± 4.67 g) was statistically not different ($p>0.05$). As early as 18 weeks, few hens started to lay her small egg (Table 2). The average weight of egg at the age of 18 to 20 weeks was 30.01 ± 2.41 g and 29.99 ± 2.10 g respectively for KUB-2_{kk} and KUB-2_{nk}. The older the hen the bigger the egg (Osei-Amponsah et al. 2015). The development of the egg size of the experimental hens followed the hypotheses. The average egg weight up to 24 weeks of age would only reach 35.7 g, which is not big enough to be hatched. According to SNI (Indonesian National Standard), the proper local day old chicken weight is 26 g as a salable chick. The size of the egg incubated can only reach as quickly as the hen reaches the age of 25 weeks by having 38 g egg. Therefore, the hens of this experiment can have their eggs incubated is at the age of 25 weeks by having the egg weight of 39 g, both for KUB-2_{kk} and KUB-2_{nk}.

The average egg weight of both groups (KUB-2_{kk} and KUB-2_{nk}) at the same age was relatively similar. At the age of 40 weeks, the average egg weight was 44.5 g and 44.8 g, for KUB-2_{kk} and KUB-2_{nk} respectively, and at the age of 44 weeks, the weight was around 46 g (Table 2). Egg size is influenced by the breed of the chicken, feed quality and amount given (Haunshi et al. 2019)reported on Indian local Aseel chicken which had a bigger size of an egg at the age of 40 weeks (48.9 g). After at 8th generation of selection the egg size was increasing 1.3 g compared to the 1st generation. Further, they mentioned that the size of an egg of 46 g was reached at the age of 32 weeks. Mengesha (2012) reported the range of egg weight of indigenous Ethiopian chicken was 42-48 g, and Ghanaian local chicken at the age of 22-48 weeks was at the range of 38 – 40.1 g (Osei-Amponsah et al. 2015). However, Ethiopian Koekoek chicken had higher egg size of 52.5 g (Shumuye et al. 2018).

Average egg production (AEP)

Average egg production (AEP_{24}) recorded for 24 weeks (20 – 43 weeks of age) of KUB-2_{kk} (101.9 ± 27.8 eggs) and KUB-2_{nk} (103.3 ± 25.9 eggs) (Table 3), did not significantly different ($p>0.05$). AEP_{24} of KUB-2 line as the average of the whole population was 102.8 ± 26.6 eggs or 61.2 ± 15.8 %. However, the AEP_{24} of KUB-2 is higher than KUB-1, which was reported (Sartika et al. 2013; Iskandar & Sartika 2014).

Variation of individual AEP_{24} of KUB-2 at 4th generation was relatively smaller (25.87%) than of KUB-1 (33.59%) reported Sartika et al. (2018). Maximum AEP_{24} of KUB-2 was 154 eggs in the first 24 weeks of production or 91.67%, whilst minimum individual AEP24 was 5 eggs or 2.98%. The KUB-2 AEP_{24} seemed to have wide individual variation, showing that chance for further selection, however when compared to some selection results on indigenous chicken from other developing countries, our KUB-2 is much better. Haunshi et al. (2019) reported that Indian Aseel selected for 8 generations produced 78.6 eggs or 53.47% during 40 weeks of observation. The production was increased from their 1st generation, which only produced 59.5 eggs or 40.48%. Matawork et al. (2019) reported that Ethiopian local chicken raised traditionally in Gena Bossa district in high, medium and low altitudes, produced 38.73, 40.45, and 36.42 eggs/hen/year, respectively.

Recently Ethiopian government exported exotic breed to improve their local chicken (Terfa et al. 2019). This program might have been based on the report of (Wondmeneh et al. 2016) who compared egg production between Horro improve breed of local chicken with modern commercial ISA Brown, crossbred of ISA Brown X Horro, and indigenous

Ethiopian breed of chicken, showed that egg production recorded for 24 weeks of those evaluated breeds, were respectively 57,1% HH; 92,2% HH; 83% HH dan 27,1%. It seems that improved Horro breed selected for 7 generations, produced eggs as much as our KUB-1 chicken.

Peak egg production of KUB-2 was 75.4% at the age of 34 weeks. Sixty percent of henday production was obtained at the age of 24 weeks persisting up until 41 weeks of age, even KUB-2_{nk} could persist more than 60% henday production up to 43 weeks of age (Figure 1). The egg production of KUB-2 chicken is much better than the egg production of KUB-1 (Sartika et al. 2013). Level of improvement of one breed will influence the level of egg production besides the quality and quantity of feed and drinking water, the length of exposure to light, management, including biosecurity, disease outbreak, the existence of parasites and another environment (Osei-Amponsah et al. 2015). As an example, Osei-Amponsah et al. (2015) added further information on local Ghana Savanah and local Ghana Forest breeds of chicken which produced egg poorly, but French Sasso breed of chicken would reach peak egg production up to 90% at the 6-8 weeks production phase.

According to individual egg production capability of KUB-2 hen at 4th generation, there were few hens (7.2% of 512 hens) produced eggs more than 80 % (Figure 2). In other words, there were 37 hens with 140.2 eggs in the first 24 weeks of age. The most number of productive hens about 28.1% of the population laid eggs more than 60% (109.8 eggs/24 weeks) and about 26.4% hens laid their eggs more than 70% (124.1 eggs) at the same period of production. There were about 21% hens of the population laid eggs less than 50 %, this means that 71% of hens laid egg more than 50%. However, since the variation of individual egg production is still relatively high (25.87%), it gives us to be able to further selection to increase egg production and production stability.

Padhi (2016) stated that selection in indigenous chicken would increase permanent production although it needed quite some time. In Ethiopian local Koekeok breed of chicken produced 180-240 eggs/hen/year (Shumuye et al. 2018). At the meantime KUB-1 just produced 160-180 eggs/ hen/year (Sartika et al. 2013), so KUB-2 produced about 102 egg/hen in the first 6 months egg production period, will need about another year selection to reach the minimum target of 180-220 eggs/hen/year.

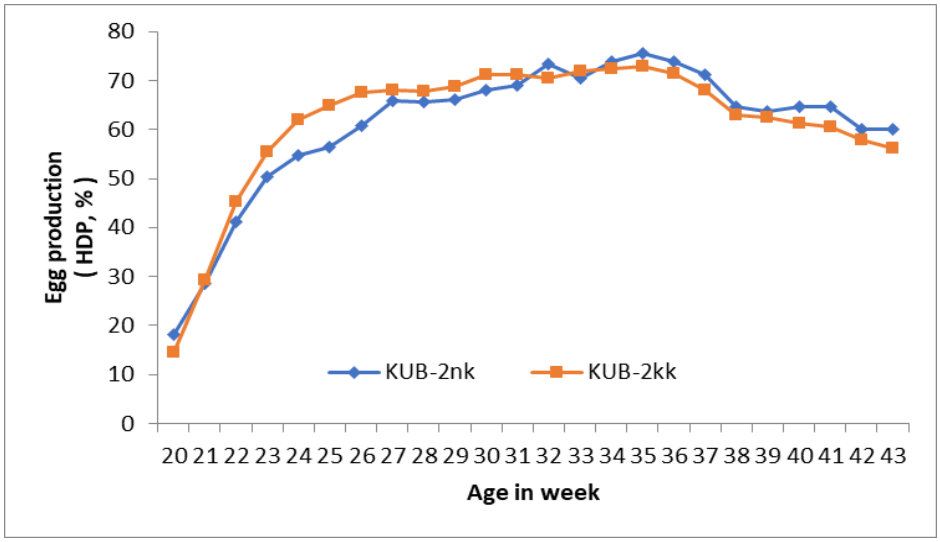
FCR and mortality

Feed Conversion Ratio (FCR) is a ratio of the weight of feed intake and the weight of egg produced in a certain time of observation. However, since the daily feed consumption was assumed around 100 g/hen, the total amount of feed consumed was calculated roughly according to number days during observation. The assumption was based on feed consumption of KUB-1 of around 80-85 g/hen/day at the level of 50% egg production (Sartika et al. 2013). Since the egg production of KUB-2 was 60%, their daily feed consumption was assumed to be 100 g/hens/day. The FCR values according to the age of the hens are presented in Table 4. The FCR figures maybe a little bit bias, but it was treated all the same to all hens, with their accurate daily individual egg recording.

Table 4. Feed conversion ratio (FCR, g feed/g egg) of KUB-2 chicken at 4th generation

Age at week		KUB-2kk[1)] (n=190)	KUB-2nk (n = 322)
20-24	Average, (g feed/g egg)	9.01±5.18	9.39±6.99
	CV (%)	57.56	74.53
25-28	Average (g feed/g egg)	4.14±0.41	3.81±0.17
	CV (%)	9.77	4.49
29-32	Average (g feed/g egg)	3.48±0.24	3.41±0.09
	CV (%)	6.77	2.82
33-36	Average (g feed/g egg)	3.13±0.14	3.17±0.05
	CV (%)	4.44	1.43
37-40	Average (g feed/g egg)	3.41±0.18	3.51±0.19
	CV (%)	5.30	5.49
41-43	Average (g feed/g egg)	3.57±0.14	3.74±0.12
	CV (%)	3.91	3.23
25-43	Average (g feed/g egg)	3.54±0.37	3.53±0.26
	CV (%)	10.43	7.34

[1)] kk = Yellow shank; nk = Non-yellow shank



kk: Yellow shank; nk: Non-yellow shank

Figure1. Egg production of KUB-2 chicken at 4th generation

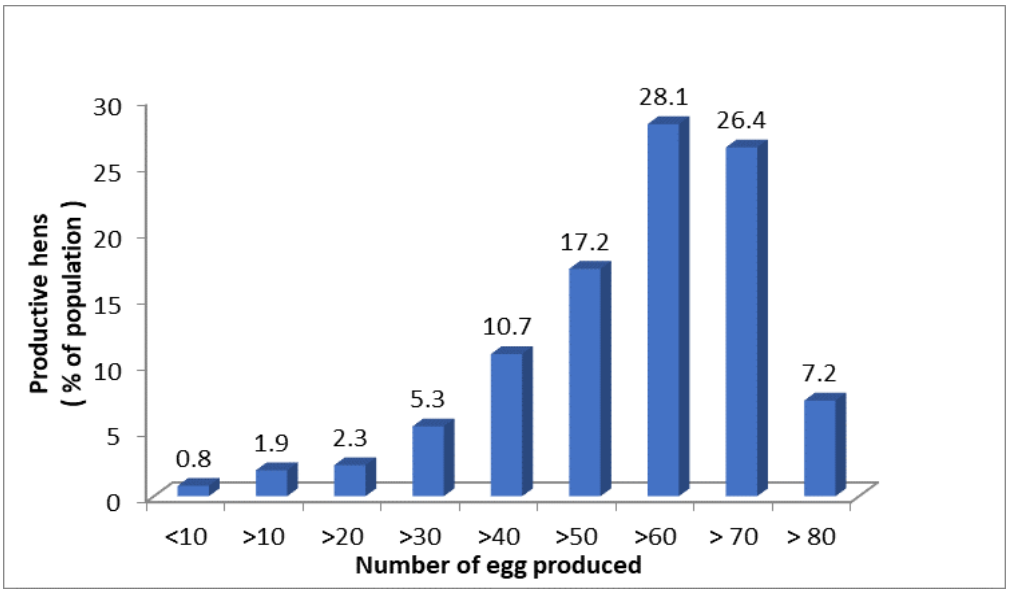


Figure 2. The egg production capacity of KUB-2 chicken at 4th generation

As it is presented in Table 4, the very poor FCR (9.4) at the age of 20-24 weeks was due to a small number of hens that laid eggs (10% of the total population), whilst the hens consumed as much as an adult hen. The number of hens which laid the egg was increasing and reached a level of 40% at the age of 24 weeks (Figure 1). The size of the egg was also smaller (30-35 g), which was not good enough for incubation. The age of 25- 43 weeks seems to be the best production phase for evaluating FCR. FCR of KUB-2 was 3.5, which is better than the FCR of KUB-1 (3.8) (Sartika et al. 2013). Wondmeneh et al. (2016) reported of FCR of 4 breeds were 3.4, 2.4, 3.3 and 7.1 respectively for improved Horro, commercial ISA-brown, crossbred ISAxHorro and indigenous Ethiopian breeds of chicken. The mortality rate during the observation was very low; there only 5 hens died of 517 hens or about 0.97% mortality.

CONCLUSION

Laying performance of KUB-2 chicken at 4th generation was much better than their parent, KUB-1. Grouping the line to the yellow shank and non-yellow shank did not influence laying performance. AFE, BWFE, and EWFE of KUB-2 were respectively 156.9

days, 1795.4 g and 31.26 g with EP_{24} was 102.8 eggs or equal to about 61.2%. At the age of 25 weeks, the size of an egg (39 g) was suitable for incubation. The FCR of 25-43 weeks of age was 3.54. Hens mortality during the experiment was 0.98%.

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Optimization of BS4 Enzyme Production with Different Substrate Thickness and Type of Trays

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ABSTRAK

Haryati T, Sinurat AP, Hamid H, Purwadaria T. 2019. Optimasi produksi enzim BS4 pada ketebalan substrat dan jenis baki yang berbeda. JITV 24(4): 158-165. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2055

Penambahan enzim BS4 pada pakan dapat meningkatkan nilai gizi bahan pakan ternak. BS4 dihasilkan oleh *Eupenicillium javanicum* BS4 dalam fermentasi substrat padat bungkil kelapa pada bioreaktor baki. Produksi enzim mungkin dapat ditingkatkan dengan aliran udara yang lebih baik melalui pori-pori pada bagian bawah baki dan ketebalan substrat. Tujuan dari penelitian ini adalah untuk mengoptimasi produksi mananase oleh *E. javanicum* BS4 dengan perlakuan ketebalan substrat dan jenis baki. Penelitian dilakukan dengan rancangan acak faktorial (2x2x3) dengan 6 ulangan. Faktor pertama adalah jenis baki (baki tidak berlubang dan baki berlubang), faktor kedua adalah ketebalan substrat (1,5 dan 3,0 cm), dan faktor ketiga adalah lama inkubasi (5 dan 7 hari). Variabel yang diamati adalah kehilangan bobot kering (KBK), aktivitas mananase dan sakarifikasi, kandungan protein terlarut, aktivitas spesifiknya dan *yield* BS4 terhadap bobot substrat. KBK pada hari ke-7 sekitar 31.43- 36.89 tertinggi ditemukan pada perlakuan baki tidak berlubang dengan ketebalan 3 cm pada hari ke-7. Hasil analisis statistik tidak menunjukkan adanya interaksi ketiga faktor, namun terdapat interaksi dua faktor pada aktivitas mananase dan *yield* pada perlakuan jenis substrat terhadap ketebalan dan terhadap lama fermentasi. Aktivitas mananase tertinggi ditemukan pada jenis baki tidak berlubang ketebalan 3 cm pada hari ke-7. Aktivitas sakarifikasi terhadap bungkil inti sawit ditemukan lebih tinggi pada jenis baki tidak berlubang dan fermentasi 7 hari, sedangkan ketebalan tidak berpengaruh. Nilai *yield* aktivitas mananase dan sakarifikasi tertinggi juga dihasilkan pada baki tidak berlubang ketebalan 3,0 cm pada waktu inkubasi pada hari ke-7. Berdasarkan efisiensi energi dan biaya, dapat disimpulkan bahwa kondisi paling optimum untuk menghasilkan enzim BS4 adalah dengan menggunakan baki tidak berlubang, ketebalan 3,0 cm dan lama fermentasi 7 hari.

Kata Kunci: *Eupenicillium javanicum* BS4, Mananase, Baki Berlubang, Fermentasi Substrat Padat, Ketebalan

ABSTRACT

Haryati T. Sinurat AP, Hamid H, Purwadaria T. 2019. Optimization of BS4 enzyme production with different substrate thickness and type of trays. JITV 24(4): 158-165. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2055

BS4 enzyme that is produced from solid substrate fermentation (SSF) on coconut cake with *Eupenicillium javanicum* BS4 in tray bioreactor has been applied as a feed additive. It increases the nutritional value of animal feedstuff. The BS4 production on SSF may be influenced by the better aeration through the perforated trays or by the thinner substrate. The aim of this research is to optimize the production of BS4 with different substrate thicknesses and types of trays. The trial was carried out using a factorial randomized design (2x2x3) with 6 replicates. The first factor was the type of trays: i.e., non-perforated and perforated tray. The second factor was the thickness of the substrate: i.e., 1.5 and 3.0 cm, while the third factor was the duration of fermentation: i.e. 5, and 7 days. The variables observed were moisture content, dry matter loss (DML), mannanase and saccharification activities, soluble protein content, their specific activities, and yield. Statistical analysis showed no interactions between the three factors, but there were interactions between types of trays and substrate thicknesses, as well as type of trays and incubation times on the mannanase activity and yield of mannanase. The results showed that DML was observed on day 7 were around 31.43- 36.89. The highest mannanase activity was observed on the non-perforated tray with 3 cm thickness on day 7. The saccharification activity towards palm kernel meal was better in the non-perforated tray on day 7 but not influenced by The yield value of mannanase and saccharification activities on a non-perforated tray with 3.0 cm thickness on day 7 was also the highest. Based on energy efficiency and the cost of production, it can be concluded that the optimum condition to produce the BS4 enzyme was observed in the non-perforated tray with 3 cm thickness and fermented for 7 days.

Key Word: *Eupenicillium javanicum* BS4, Mannanase, Perforated tray, Solid Substrate Fermentation, Thickness

INTRODUCTION

The feed is the largest contributor to the cost of livestock production, especially when some of the feed ingredients are imported. Imported feed ingredients are normally expensive which makes farmers difficult to gain profit. Farmers may use the agricultural industry by-products such as coconut meal (CM) and palm kernel meal (PKM) in order to reduce the feed price. PKM a by-product obtained in the production of palm kernel oil has been used as an ingredient for poultry feed. However, the inclusion of PKM in poultry feed is still low due to the high content of crude fiber. Its fibers, such as mannan and cellulose caused low digestibility of nutrients (Ketaren et al. 1999). Fibers contained in the feed can be decomposed by the addition of hydrolytic enzymes thus increasing the nutrient digestibility of feed material (Kuhad et al. 1997).

The use of PKM and CM in the poultry feed is still not optimal, due to high fiber content and low energy and protein content. The crude fiber in the PKM is 11.9 – 15.3% (Sinurat 2012) and the crude fiber in the CM is about 23.5-25.5 % (Jaelani 2007). The fiber content in the PKM and CM mostly composed of hemicellulose and dominated by manan and galactomannan (Mairizal 2013). These components of mannan and galactomannan in the PKM and CM are not digested in poultry and reduces the feed nutritional value (Shimizu et al. 2015). The manan and galactomannan are anti-nutrients which can increase the viscosity of the digesta in the gut producing the reduction of absorption of nutrients in the intestine (Mairizal 2013)).

Hydrolysis of mannan into mannooligosaccharides and mannose can be performed using mannanase enzymes. The mannanase enzyme can be applied to increase the nutritional value of poultry feed by hydrolytic activity thus increasing the solubility of crude fiber in the feed (Sigres & Sutrisno 2015). One of the microorganisms that can produce mannanase enzyme is *Eupennicillium javanicum* BS4. The mold has been selected because it produces high-level mannanase and can be well grown in submerged culture containing coconut cake (Purwadaria et al. 2003). In producing enzyme by fermentation, conditions such as temperature, moisture content, aeration, and substrate particle size are important factors. In the solid substrates fermentation, the production process can be affected by several factors such as substrate thickness and aeration condition. Substrate thickness may affect moldy mycelial growth. The thicker the substrate, the mycelium growth in the inside will be inhibited and decreases the production of mannanase enzyme. Aeration is a factor that plays a role in the fulfillment of oxygen demand and together with the release of CO_2 and heat during the fermentation process. A good aeration process will determine the growth rate of microorganisms and may increase the production of enzymes during the fermentation process (Ab Rashid et al. 2012). The enzyme production study by (Maximilian 2017) using a medium-scale tray bioreactor with a capacity of 96 kg showed that the best condition of mannanase enzyme production was observed on a tray with a thickness of 1 cm for 5 days incubation. The thin substrate may support maximum growth of the mold but the total enzyme produce per batch may less. In order to increase the production of mannanase enzymes, a study was designed by increasing the substrate thickness and tray-type in mid-scale tray bioreactors. The presence of holes in the tray is expected to increase airflow on the lower surface so as to reduce airflow resistance to thick substrates and improve metabolism and mold growth during the fermentation process.

MATERIALS AND METHODS

Materials

The substrate materials used for the fermentation was coconut meal, supplied by local feed mill in Bogor. *E. javanicum* BS4 fungi, a collection of Indonesian Research Institute for Animal Production was used as an inoculum to produce the enzyme. The fungi were first grown on Potato Dextrose Agar (PDA) medium for 5 days at room temperature.

Inoculum and fermentation process

Inoculum was prepared by the addition of sterile saline into freshly grown PDA slants. Submerged fermentation was carried out by inoculated 2.5 mL of spore suspension into 250 ml containing 50 ml of potato sucrose broth. The flask was incubated at a rotary shaker at 120 rpm, 28º C for 5 days. Coconut meal as substrate was steamed for 15 minutes at 121ºC, cooled, mixed with mineral mixtures from Mandels minimal medium (Maximillian 2017) and then mixed with 10% of *E. javanicum* BS4 inoculum prepared in the submerged culture. The substrate was put on two (2) kinds of stainless steel trays (tray with holes and trays without holes at the bottom). The amount of the substrate put on the tray was either thin (1.5 cm) or thick (3.0 cm). The fermentation process was carried out in a stainless steel bioreactor. The temperature in the bioreactor was set at 28 ºC and humidity (Rh) of 85 %.

Sample collection and enzyme extraction

Samples were collected at 5 or 7 days and determined for the moisture content and dry matter

loss (DML). The fermented product was extracted in 10x 0.2 M Na-acetate buffer to obtain the enzyme. The mannanase and saccharification activities and their specific activities of the enzyme were determined. DML was calculated by measuring the reduction of DM weight of the substrate before fermentation towards DM weight of fermented products. The yield of enzyme activities towards kg substrate was determined by multiplying the activity in DM to the DM of the product. The DM loss was already considered.

Mannanase activity assay

Mannanase activity was assayed by mixing 0.5 mL of an appropriate diluted enzyme solution with 0.5 ml of 0.5% locust bean gum in 50 mM acetic buffer (pH 5.8) at 40 °C for 30 min. The reaction was stopped by the addition of 1.5 mL dinitrosalicylic acid (Miller 1959). After 5 minutes of boiling, the amount of reducing sugars was determined spectrophotometrically at 540 nm. Mannose was used as a standard. One unit mannanase was defined as the amount of the enzyme in g DM which liberates 1 µmol mannose per minute under the assay conditions.

Saccharification activity

The saccharification activity of the enzyme was determined by measuring the amount of reducing sugars produced from the decomposition of carbohydrates. In this experiment, palm kernel meal (PKC) was used as a source of carbohydrate. Saccharification activity was calculated as U/g DM, while specific activity saccharification of the enzyme calculated as U/mg protein. One unit of activity is the amount of enzyme in g DM that liberates 1 µmol of glucose per minute under assay condition.

The specific activity of the enzyme.

The specific activity of the enzyme was determined towards soluble protein concentration. Protein was determined by the method of Bradford (1976) and Bovine Serum Albumin (BSA) was used as a standard. The measurement of the protein content was expressed in mg protein per g dry weight and specific activity of the enzyme calculated in U/mg of extracellular proteins.

Statistical analyses

All enzyme activities and yields with six replications were analyzed statistically by analyses of variance (ANOVA) in 2 x 2 x 3 factorial design. Differences between treatments was tested by the least significant difference (LSD) when the ANOVA was significant at $P<0.05$ (Steel & Torrie 1980).

RESULTS AND DISCUSSION

Moisture content in the course of fermentation

The moisture content of the substrate after fermentation decreased as the duration of incubation increased in all treatments (Figure 1). The most stable moisture content is detected on the treatment of non-perforated tray with 1.5 cm thickness which is in the range of 50-60%. In the perforated trays with 1.5 cm and 3.0 cm substrate thickness, the moisture content was lower on the 5th and 7th day of incubation, i.e., in the range of 35-45%, although the initial moisture content was similar for all treatments. The reduction occurred due to the high flow of aeration produced an excess of evaporation. In the common aerobic solid substrate fermentation moisture content is increasing towards the duration of fermentation, since respiration produces water molecules. It is possible that the amount of water evaporation was higher than that of the water formation. The excess of evaporation would not occur if control in the bioreactor was working well. The addition of the holes in the perforated trays caused more evaporation which produced very low moisture content on the 5th and 7th days (Figure 1).

In the solid substrates fermentation, water content is one of the important biological factors for fungi growth (Chang & Webb 2017). Moisture content influenced the growth of mold and production of BS4 enzymes (mannanase and saccharification activities). The mycelia already grew in the beginning, when the moisture content was still 50 to 60%. In the late fermentation (5 to 7 days fermentation) the mycelia including the enzyme should have produced especially in the SSF process with had a lot of substrates. However, the loss of excessive moisture reduces the nutrient solubility in the substrate, thus interfering with the mold metabolism activity (Stark & Firestone 1995) including growth and enzyme production. In the top of that data with the moisture content reaching 35% on the perforated trays indicated the condition of the substrate was close to the minimum moisture content for the metabolic activity. Therefore, the enzyme activities of this treatment were the lowest.

Dry matter losses (DML) in the course of fermentation

DML in all trays type and substrate thickness treatments increased when the duration of fermentation increased from day 5 to day 7 (Figure 2). The highest DML was observed in the non-perforated

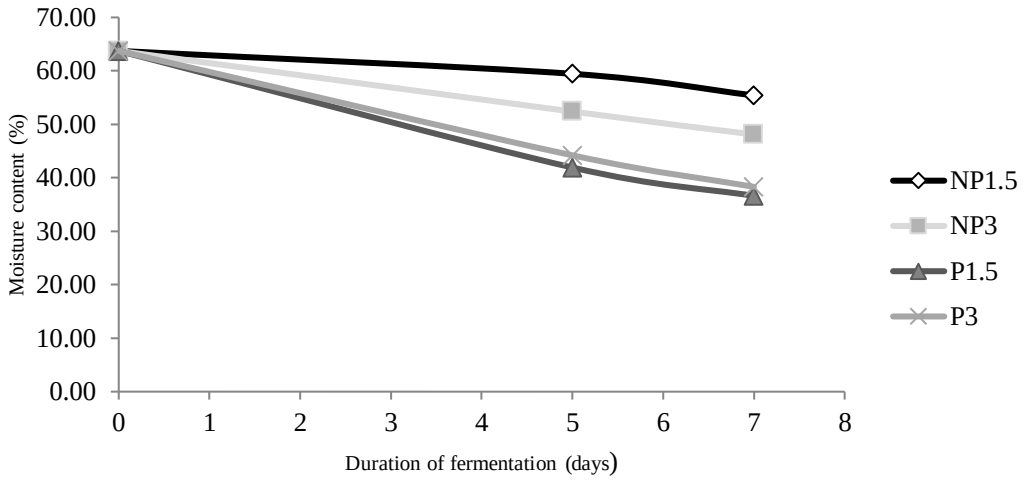

Figure 1. Moisture content of the substrate after fermented on different kind of trays and substrate thickness
*NP: non-perforated; P: perforated

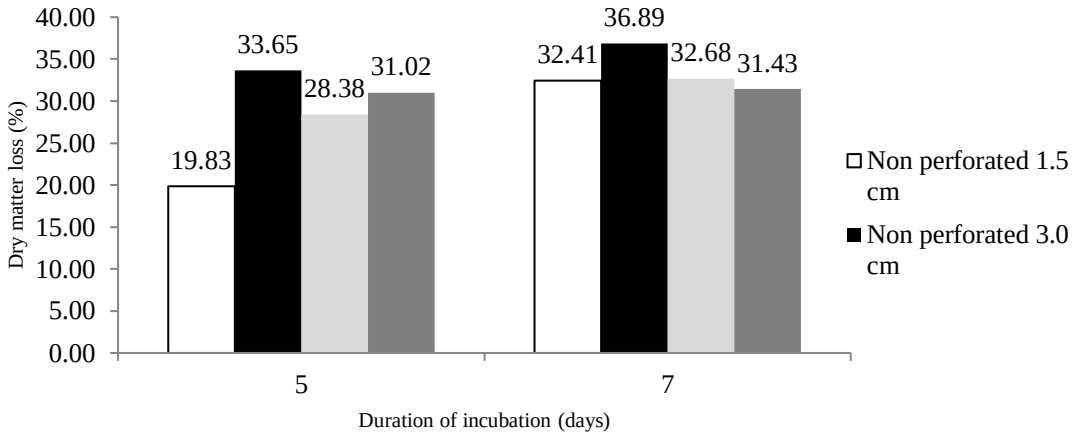

Figure 2. Dry matter loss during the fermentation process

Table 1. Mannanase activity of fermentation product with *E. javanicum* incubated on different tray type and substrate thickness

Type of trays	Substrate thickness (cm)	Mannanase act (U/g DM)
Non perforated	1.5	$217.72^{a(*)}$
	3.0	499.65^{c}
Perforated	1.5	346.44^{ab}
	3.0	387.11^{bc}

(*) Different superscripts indicate statistically significant difference within column P< 0.01

Table 2. Mannanase activity of fermentation product of *E. javanicum* BS4 incubated on different tray type and duration of fermentation

Type of tray	Duration of fermentation (day)	Mannanase (U/g DM)
Non perforated	5	$255.34^{a(*)}$
	7	487.67^{b}
Perforated	5	325.90^{a}
	7	411.35^{ab}

(*) Different superscripts indicate statistically significant difference within the column P< 0.01

trays with a thickness of 3.0 cm on the 7th day which reached 34% losses. Fermentation in non-perforated trays with 1.5 cm thickness on day 5 showed the lowest (19.83%) DML, but it increased to 32% when the fermentation performed for 7 days. Fermentation in perforated trays with a thickness of 1.5 cm and 3.0 cm shows DML of 31-32% which almost equals those in non-perforated trays with 3.0 cm thickness.

Dry matter losses were increasing during the fermentation process due to the degradation of carbohydrates into CO_2, H_2O, and energy. Therefore, the DML data are always parallel with the growth specificity. The DML values are also related to the water content of the fermented product. In solid substrate fermentation, the moisture content is one of the important biological factors for mold growth (Chang & Webb 2017). In general, the water content of solid substrate fermentation ranges from 30 to 85% (Raimbault 1998). It was already discussed that excessive moisture losses could reduce nutrient solubility in the substrate, thereby disrupting mold growth and reducing DML (Stark & Firestone 1995).

The activity of mannanase produced

There was no significant ($P>0.05$) interaction between the type of trays, substrate thickness, and duration of fermentation on the activity of mannanase produced. However, there were significant ($P<0.05$) interactions between type of trays and substrate thickness, as well as the type of trays and incubation time in the mannanase activity. The mannanase activity produced was affected by substrate thickness when the fermentation was performed in the non-perforated trays, while it was not significantly ($P>0.05$) different when the fermentation was carried out in the perforated trays (Table 1). The highest mannanase activity (499.65 U/g DM) was produced when the fermentation was carried out in the non-perforated trays with 3.0 cm substrate thickness and the lowest (217.72 U/g DM). The highest mannanase activity obtained in the thicker (3 cm) substrate is not in accordance with the hypothesis stated by Ab Rashid et al. (2012) who found that the thinner (0,5 cm) substrate in the trays produced higher mannanase activity than the thicker (1.0 and 1.5 cm) substrate. The inoculum used for the fermentation was *Aspergillus niger* USM F4 and the substrate was palm kernel cake.

The duration of fermentation significantly ($P<0.01$) affected the mannanase activity produced especially for the non-perforated tray (Table 2). The mannanase activity produced on non-perforated was higher when the fermentation performed for 7 days (487.67 U/g DM) compared to those fermented for 5 days (255.34 U/g DM). When the activity was compared in the perforated tray 7 days incubation was higher 26.2%, even though it was not significantly different (411.35 vs 325.90 U/g DM). Non significantly data has resulted from the variation that occurred between sample replication. This indicated that the optimum duration of fermentation by using *E. javanicum* is 7 days. This result is in line with DML data, where on non-perforated tray and 7 days fermentation the DML was higher. The optimal duration of fermentation was much influenced by a kind of inoculum and fermentation process. (Sae-Lee 2007) reported that the optimal duration of solid substrate fermentation to produce mannanase was observed at 6 days for *Aspergillus niger*, while there were for 4 and 7 days for *A. oryzae* and *Penicillium* sp. respectively. The same optimal duration was observed with BS4 fermentation and *Penicillium* sp., although the substrate in the manuscript is PKM and the fermentation was carried out in the flask. *Eupenicillium* is very related to *Penicillium*.

Mannanase specific activity, saccharification activity and its specific activities of enzymes produced

The saccharification activities of the enzyme produced are shown in Table 3. Statistical analyses showed that there were no significant ($P>0.05$) interactions between all factors (the type of tray, substrate thickness, and duration of fermentation) in this experiment. The main factors such as type of trays and duration of fermentation but not the substrate thickness significantly ($P<0.05$) affect the saccharification activity of the enzyme produced. Fermentation in non-perforated trays produced significantly ($P<0.05$) higher saccharification activities (85.85 U/g DM) compared to fermentation in perforated trays (56.74 U / g DM). However, the specific mannanase activity (the mannanase activity per mg protein) and the specific saccharification activity (the saccharification activity per mg protein) of enzyme produced in both types of trays were not significantly different ($P>0.05$). Duration of fermentation also significantly ($P<0.05$) affect the saccharification activities but not the specific mannanase and specific saccharification activities. Fermentation for 7 days produced significantly ($P<0.05$) higher saccharification activities (85.45 U/g DM), compared to fermentation for 5 days (57.14 U/g DM).

Saccharification activity shows the activity of enzymes that can break down carbohydrate complex or fibers components in palm kernel cake (PKC) becoming more simple carbohydrates. The higher the saccharification activity indicates that the enzyme has a higher possibility to be applied in animal feed containing high fiber feed ingredients such as PKC. The saccharification activity measured in this experiment was not only due to the presence of mannanase enzymes but also by the presence of other enzymes such as cellulase and xylanase. Besides mannan, PKC also contains other complex

Table 3. Saccharification, specific mannanase, specific saccharification activities of fermentation products of BS4 with tray type, substrate thickness, and duration of fermentation

Treatment		Saccharification Activity (U/g DM)	Specific Mannanase Activity (U/mg)	Specific Saccharification Activity (U/mg)
Type of trays	Non perforated	85.85[b(*)]	8.28	2.48
	Perforated	56.74[a]	10.54	1.81
Substrate thickness	1.5 cm	69.67	9.07	2.65
	3.0 cm	72.65	9.69	1.72
Duration of fermentation	5 days	57.14[a]	8.21	1.77
	7 days	85.45[b]	10.61	2.51

(*) Different superscripts in same column indicate a statistically significant difference between a column in the treatment compared P<0.05.

Table 4. The yield of mannanase activity as affected by the type of trays and substrate thickness

Type of trays	Thickness (cm)	Mannanase (U/kg)
Non-perforated	1.5	155, 239[a]
	3.0	320, 917[b]
Perforated	1.5	245, 490[ab]
	3.0	266, 265[b]

(*) Different superscripts indicate statistically significant difference within column P< 0.01

Table 5. The yield of mannanase activity as affected by the type of trays and duration of fermentation

Type of trays	Duration of fermentation (days)	Mannanase (U/kg)*
Non-perforated	5	176, 067[a]
	7	315, 151[b]
Perforated	5	233, 956[ab]
	7	279, 687[ab]

(*) Different superscripts indicate statistically significant difference within column P< 0.01

Tabel 6. The yield of saccharification activity of the enzyme produced in a different type of trays, substrate thickness, and duration of fermentation

Treatments	Saccharification yield (U/kg substrate)
Type of trays:	
Non perforated	57, 655[b]
Perforated	39, 841[a]
Substrate thickness:	
1.5 cm	49, 993
3.0 cm	47, 711
Duration of fermentation:	
5 days	41, 368[a]
7 days	56, 129[b]

(*) Different superscripts indicate a statistically significant difference between treatment compared P< 0.05

carbohydrates such as cellulose and xylan (Abd-Aziz et al. 2009).

It is very possible that the specific activity of mannanase and saccharification were not significantly different, although the activity in the dry matter was different. Those data occurred due to the increase and the decrease of activity are parallel to the protein concentration. The one not expected is the specific saccharification activity, since it implies more enzymes than that mannanase. It seems that the protein concentration also parallels with each enzyme activity. This conclusion might be confirmed if the purification of every enzyme was carried out.

Yield value of mannanase and saccharification activities towards substrate.

The yield value is calculated to evaluate the efficiency in producing enzyme activities including both mannanase and saccharification on a similar amount of substrate in order to decide the most optimum method of producing the enzyme. The evaluation already considered DML. Statistical analysis on yield of mannanase activity showed that there were interaction between types of trays and substrate thickness, as well as type of trays and duration of fermentation, while that of saccharification activity showed that there was no interaction between all factors (type of trays, substrate thickness, and duration of fermentation) in this experiment (Table 4, 5, and 6). Following data of mannanase activity, the highest yield of mannanase activity was observed in the non-perforated tray at 3 cm substrate thickness and 7 days duration of fermentation. The treatment shows the most efficient enzyme production, although it also resulted in the highest DML. The high activity covered the loss of the substrate.

The saccharification yield was significantly affected by the main factor effect of type of trays ($P<0.01$) and the duration of fermentation ($P<005$), but not significantly ($P>0.05$) affected by substrate thickness. The enzyme produced in non-perforated trays yields higher saccharification activity compared with those produced in perforated trays (57.655 U/kg vs 39.841 U/kg substrate). Fermentation carried out for 7 days produced an enzyme with significantly ($P<0.05$) higher saccharification yield (56.129 U/kg substrate) than that produced for 5 days (41.368 U/kg substrate). The yield of saccharification activity from 3 cm thickness was similar to the 1.5 cm, therefore on the base of BS4 enzyme activities and yield toward substrate it can be concluded that BS4 production was best carried out in the non-perforated tray with 7 days duration of fermentation and 3 cm thickness.

In the fermentation process, the value of $Y_{p/s}$ or yield of a product towards substrate is generally calculated in the same unit, as occurs in ethanol fermentation with a yield of 0.44 g/g (Mathew et al. 2015). The value is always below 1, especially if both compounds are built from similar molecules like glucose as the substrate and ethanol as the product. Both molecules contain carbon, oxygen, and hydrogen. Although the $Y_{p/s}$ is lower than 1, the process is economic. In this study, efficiency is evaluated based on the comparison of enzyme activity against the substrate (Unit/kg substrate). Enzyme activity was measured as the product since it works as a biocatalyst. The yield value was much higher than 1, however, it does not imply economic benefit. The economic value should consider all the costs of the production and the effectivity of the enzyme in the application. The yield data in the experiment only show the efficiency of the substrate for each treatment.

CONCLUSION

The type of trays and substrate thickness affects the growth of *E. javanicum* BS4, thus affecting the production of enzymes in medium-scale tray bioreactors. The use of perforated trays may lead to excessive oxygen transfer on the substrate so that the moisture content becomes very low and dry. The thicker substrate was better to maintain the water content during the fermentation process than the thinner substrate. Based on mannanase and saccharification activities as well as the yield of activities towards the substrate, BS4 production was best produced on a non-perforated tray with 3.0 cm thickness for 7 days fermentation.

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The Use of Coffee Husk as Napier Grass Substitution and Its Effect on Madura Cattle Performance

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ABSTRAK

Sudarman A, Listiawan GB, Khotijah L. 2019. Penggunaan kulit kopi sebagai pengganti rumput gajah pada performa sapi Madura. JITV 24(4): 166-172. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2006

Penelitian ini bertujuan untuk mempelajari kinerja produksi sapi Madura, dengan pemberian kulit kopi sebagai sumber serat pengganti hijauan rumput dalam ransum. Dua puluh ekor sapi Madura jantan umur sekitar 1,5 sampai 2,0 tahun dengan bobot awal 165-190 kg dibagi secara acak menjadi empat macam perlakuan pakan. Pakan yang diberikan setiap perlakuan yaitu R0= konsentrat 90% + kulit kopi 0% + rumput gajah 10%; R1= konsentrat 90% + kulit kopi 3,33% + rumput gajah 6,67%; R2= konsentrat 90% + kulit kopi 6,67% + rumput gajah 3,33%; R3= konsentrat 90% kulit kopi 10% + rumput gajah 0%. Pakan yang diberikan sebesar 3% bahan kering dari bobot badan. Sapi dipelihara dalam kandang secara individu selama dua bulan. Variabel yang diamati meliputi konsumsi bahan kering, efisiensi pengggunaan pakan, pertambahan bobot badan harian, kecernaan pakan dan keuntungan. Data yang diperoleh dianalisis dengan sidik ragam dari rancangan acak kelompok yang dilanjutkan dengan uji jarak berganda Duncan. Hasil penelitian menunjukkan bahwa konsumsi bahan kering, efisiensi pengggunaan pakan, pertambahan bobot badan harian dan kecernaan pakan sapi pada setiap perlakuan pakan tidak berbeda nyata (P>0,05). Rataan konsumsi bahan kering dan penambahan berat badan harian untuk R0, R1, R2, dan R3 masing-masing adalah 6,01; 5,84; 5,73 dan 5,62 kg/ekor/hari dan 0,88; 0,87; 0,84 dan 0,93 kg/ekor/hari/hari. Sedangkan rata-rata efisiensi pakan dan kecernaan DM untuk R0, R1, R2, dan R3 masing-masing adalah 14,64, 14,89, 14,65 dan 16,54% dan 84,82, 84,37, 83,47 dan 83,30%. Kesimpulannya adalah penggunaan 10% kulit kopi sebagai sumber serat pengganti rumput gajah dapat digunakan tanpa mengganggu performa sapi Madura dan memberikan nilai IOFC cenderung lebih tinggi sehingga memberikan keuntungan cenderung lebih besar pada program penggemukan.

Key words: Kulit Kopi, Pemanfaatan Pakan, IOFC, Performa Sapi Madura, Rumput Gajah

ABSTRACT

Sudarman A, Listiawan GB, Khotijah L. 2019. The use of coffee husk as napier grass substitution and its effect on Madura cattle performance. JITV 24(4): 166-172. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2006

This research aimed to evaluate the performance of fattened madura cattle fed on coffee husk as a source of fibre to substitute grasses. Twenty Madura steers aged approximately 1.5 to 2.0 years with initial weight of 165-190 kg were divided randomly into four different experimental diets, namely R0= 90% concentrates +0% coffee husk +10% napier grass, R1= 90 % concentrates +3.33% coffee husk +6.67% napier grass, R2 = 90% concentrate +10% 6.67% coffee husk +3.33% napier grass, R3 = 90% concentrates 90% + 10% coffee husk +0% napier grass. The feed was given at 3% body weight of dry mater. The cows were kept in individual pen for two months. Variables measured were dry mater intake, feed efficiency, average daily gain (ADG), digestibility of feed and income over feed cost. Data obtained were analyzed using analysis of variance based on randomized block design followed by Duncan's multiple range test. Results showed that dry matter intake, ADG, feed efficiency and feed digestibility of cattle on each treatment of the feed were not significantly different (P > 0.05). Average of dry matter intake and daily gain for R0, R1, R2, and R3 were 6.01, 5.84, 5.73 and 5.62 kg/head/day and 0.88, 0.87, 0.84 and 0.93 kg/head/day respectively. While the average of feed efficiency and DM digestibility for R0, R1, R2, and R3 were 14.64, 14.89, 14.65 and 16.54 % and 84.82, 84.37, 83.47 and 83.30 %, respectively. It is concluded that the used of 10% coffee husk as a source of fibre for substitution of napier grass can be used without negative effect on madura's cattle performances and tend to give higher values of IOFC on fattening program.

Key Word: Coffee Husk, Feed Utilization, IOFC, Madura Cattle Performance, Napier Grass

INTRODUCTION

Beef as a source of animal protein have a role in fulfilling community nutrition to support the development of Indonesia's human resources. Along with increasing population, income, and increasing public knowledge of the importance of animal protein, demand for beef is also increasing. Direktorat Jenderal

Peternakan dan Kesehatan Hewan (2017) reported that the Indonesian beef cattle population in 2015 was 15,419,718 heads and in 2016 became 16,004,097 heads or increased by 1.22%. While, beef production in 2015 was 506,661 showed an increase by 2.28% from 2016 (518,484 tons). This is an opportunity for farmers to develop their cattle farm business.

Livestock performance and efficient use of feed are the main indicators of the success of a livestock business. To produce cows with good production performance, feed with nutrient content that can meet the livestock production requirements is needed. In the cattle fattening program, feeding concentrates as the main feed with sufficient nutrient content can produce good cattle performance. The addition of Napier grass as a source of fiber is also important because it can be used as a deterrent to the occurrence of metabolic disorders in cattle. Availability of grass as a source of fiber is not always sufficient throughout the year. Forage production is usually high during the rainy season. However, in the dry season many farmers face difficulties in obtaining forage, especially grass because it is scarce and expensive.

Feed sources of fiber other than grass, such as coffee by-product (mainly coffee pulp and husk), can be used as alternative feed when forage is difficult to obtain. Direktorat Jenderal Perkebunan (2016) reported that coffee production in Indonesia was 639 305 tons. Widyotomo (2013) reported that every ton of wet fruit will produce 200 kg of dry coffee pulp. This is potential to be used as fiber source for animal feed during the dry season.

Use of coffee by-products such as pulp and husk as a single feed for livestock is not recommended, due to the presence of antinutrient and certain nutrient deficiencies. Therefore, coffee by-products must be mixed with other feed ingredients when given to livestock. Badarina et al. (2013) reported that coffee husk can be used up to 20% in the ration after being fermentated by *Pleurotus ostreatus*. Increasing level of coffee hull addition into concentrate for dairy heifer reduced microbial protein synthesis 0.687 g/day for each percentage unit of coffee hull added in the concentrated (de Souza et al. 2010). Reduction in microbial protein synthesis can reduce weight gain in heifers fed coffee hulls. Didana (2014) reported that in beef cattle show a decrease in feed intake and weight gain directly related to the level of coffee pulp in the diet. On the contrary, Nunez et al. (2015) reported that dry coffee pulp in diets could be supplemented to ruminant diets for supporting milk and meat production without any adverse effect on their health.

The purpose of this study was to evaluate the effect of coffee husk use as a substitution for grass in cattle rations on the production performance of cattle and the value of income over feed cost (IOFC).

MATERIALS AND METHODS

This study was conducted from April to July 2017 in small holder farm at Tegalwaru Village, Ciampea District, Bogor Regency. Proximate analysis of feed was carried out at the Laboratory of Biological Resources and Biotechnology Research Center, Institute for Research and Community Empowerment, Bogor Agricultural University.

In this study 20 male madura cattles about 2 years old with an initial body weight ranged between 169 - 186 kg were used and placed in individual cages equipped with feed and drinking water containers. The feed given were concentrate, coffee husk, napier grass (Pennisetum purpureum) and multivitamins. Drinking water from the well was given ad libitum.

The experimental feeds were made by mixing concentrates, coffee husk and napier grass with a proportion determined for each treatment in each cow feed container.. Before offered, napier grass was chopped using a chopper machine. The compositions of concentrates are presented in Table 1. Price and nutrient contents (based on proximat analysis) of feed used in this experiment were shown in Table 2.

Maintaining experimental animals

Animals were adapted to experimental feed and environment for two weeks. After that, the cattle were weighed and given dewormer medicine Kalbazen (10-15 ml / head). During experiment weighing of animal were carried out every two weeks. Animals were kept in an intensive fattening program on individual cages, each of which is equipped with food and drinking water containers and maintained for two months. Feeds were given as much as 3% of cattle body weight on dry matter basis with concentrates and fiber sources ratio of 90:10. The experimental feeds were given at 6:30 a.m., 12:00 a.m, and 19:00 p.m. Feed refusals were weighed in the morning at 6:00 to calculate feed intake. The nutrient content of the experimental feed is presented in Table 3.

Faecal collection

Faecal collection was carried out at the end of feding trials for seven consecutive days to determine the digestibility of dry matter, crude protein, and crude fiber in each treatment. Each cage was given a barrier so that the collected faeces did not mix for each treatment. The collected faeces were then weighed and taken about 10% of the total faeces everyday and then dried under the sun as samples. Samples were then stored in plastic bags and labeled. Total samples that have been dried were then put into the oven 60 °C for

Table 1. Composition of feed ingredient in concentrate

Feed ingredient	Proportion (%)
Cassava flour waste	30
Rice bran	9
Pollard	8
DDGS*	8
Molasses	11.50
Soy bean meal	5
Coconut meal	12.50
Palm oil cake	9
Soya full fat	4
Urea	0.75
NaCl	0.75
CaCO3	1
Premix kalbe**	0.5
Total	100

*DDGS (Destillers Dried Grain with Solubles)
** Each 5 kg contains: vitamin A 10,000,000 IU, vitamin D 2,000,000 IU, vitamin E 3,000 mg, vitamin B2 5,000 mg, vitamin B12 5,000 mcg, vitamin B1 1,000 mg, vitamin K 1,000 mg, choline chloride 100,000 mg, nicotinamide 7,500 mg, DL-methionine 100,000 mg, foloc acid 500 mg, D-lysine HCL 75,000 mg, ferrous sulfate 25,000 mg, manganese sulfate 50,000 mg, magnesium sulfate 34,000 mg, cupric sulfate 5,000 mg, Ca-d-panthotenate 2,500 mg, zinc sulfate 10,000 mg, potassium iodice 100 mg, antioxidant and carrier q.5. ad 5 kg.

Table 2. Proximate analysis and price of feed ingredients used in this study

Feed	Price (Rp)*	Ash	CP	EE	CF	NFE	TDN**
Concentrate	3 395	16.57	17.00	4.00	10.98	51.45	67.12
Coffee husk	1 381	9.20	8.22	0.58	31.62	50.38	51.57
Napier grass	3 529	14.26	11.86	2.05	29.00	42.83	52.00

Results of laboratory analyzes of Research Center for Biological Resources and Biotechnology of IPB (2017)
*Price based on feed dry matter.
**TDN was calculated according to Hartadi et al. (1980)

Table 3. Nutrient content (% DM basis) of experimental diets

Nutrients	Treatment Diets*			
	R0	R1	R2	R3
Ash	16.34	16.17	16.00	15.83
Crude protein	16.48	16.36	16.23	16.12
Ether extract	3.81	3.75	3.71	3.66
Crude Fiber	12.78	12.87	12.96	13.04
NFE	50.59	50.85	51.10	51.35
TDN	65.61	65.59	65.58	65.56
E:P ratio	3.98	4.01	4.04	4.07

* R0 = 90% concentrate + 0% coffee husk + 10% napier grass, R1 = 90% concentrate + 3.33% coffee husk + napier grass 6.67%, R2 = 90% concentrate + coffee husk 6.67 % + napier grass 3.33% and R3 = 90% concentrate + 10% coffee husk + napier grass 0%.

48 hours. The samples were then milled and analyzed for the dry matter, crude protein, and crude fiber content.

Experimental design and data analysis

Experimental design applied was a randomized block design (RBD) with five treatments and five groups of livestock based on body weight as replication. The grouping of livestock in this study were: 165-170 kg, 171-175 kg, 176-180 kg, 181-185 kg and 186-190 kg. Feed treatment applied in this study were: R0 = 90% concentrate + 0% coffee husk + 10% napier grass, R1 = 90% concentrate + 3.33% coffee husk + napier grass 6.67%, R2 = 90% concentrate + coffee husk 6.67 % + napier grass 3.33% and R3 = 90% concentrate + 10% coffee husk + napier grass 0%

Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test if there were differences in mean treatment. Variables observed in this study included: dry matter intake, daily body weight gain, efficiency of feed utilization, digestibility of dry matter, crude protein, crude fiber and income over feed cost (IOFC). The IOFC values were obtained by substracting the amount of income obtained from seling of cattle (body weight gain multiplied by price) with the cost of feed.

RESULTS AND DISCUSSION

Nutrient intakes, weight gain and feed utilization efficiency

Nutrient intakes, body weight gain and feed efficiency data are shown in Table 4. Nutrient intake is required for the purpose of maintenance and production (growth, milk production and pregnancy). Different fiber sources in the rations had no effect (P> 0.05) on dry matter intake. This may indicated that the rations with fiber sources from coffee husk or napier grass has similar palatability (Table 4). Daily weight gains were also not different (P> 0.05) among the treatments which were in line with the amount of dry matter intake.

Dry matter intake for all treatments has given cattle body weight gain ranging from 0.84 to 0.93 kg / day. This means that intake of DM at present experiment has been sufficient for cattle in the fattening phase. The average body weight of cattle during the study for R0, R1, R2 and R3 were 206.4, 205.6, 204.6 and 206.1, respectively. Meanwhile percentage of DMI/LW for R0, R1, R2 and R3 are 2.91, 2.85, 2.79, and 2.72%, respectively. According to Kearl (1982) for cattle in tropical region with live weight of 200 kg and expected weight gain of 0.75 – 1.00 kg should have percentage of DMI/LW around 2.7 – 2.8%. Except R0, other treatments (R1, R2 and R3) were in accordance with those recommended by Kearl (1982).

Experiment with different feed, Rab et al. (2016) showed that Madura cattle fed diet (85% concentrate + 15% soybean pod) with dry matter content of 72.2%, crude protein 14%, crude fibre 17.1%, NFE (nitrogen free extract) 55.8% and TDN 71% resulting in dry matter intake higher (6.92 ± 0.24 kg/head/day) than present experiment. However, it is further reported that daily gain obtained was only 0.73 kg/head/day which was lower than those obtained in the present experiment. This may indicated that the feed quality used in experiment of Rab et al. (2015) was lower than that in the present experiment as their experimental diet had crude protein 14% and crude fibre 17.1%, on the other hand the experimental diet in this study contained crude protein ranged 16.12 – 16.48% and crude fibre ranged 12.78 – 13.04. It may also be concluded that between these two agriculture wastes, coffe husk can be used better as feed ingredient for beef cattle than soybean pod. Coffee husk, in this experiment, had crude protein and crude fibre of 8.22 and 31.62%, respectively, meanwhile soybean pod had crude protein and crude fibre content of 5.5 and 35.4%, respectively (Wiryawan et al. 2017).

Intake of other nutrients in this study had the same pattern as dry matter intake and statistically all were not significantly different (P> 0.05). Crude protein intake of each feed were relatively similar in all treatments which are directly proportional to dry matter intakes. According to Tuwaidan et al. (2015) the crude protein content in feed greatly determines the dry matter intake. Protein requirement, that will affect the intake, vary according to the body size of the animal, its physiological status, management system, feed resources, and expected performance. Among those factors, only feed resources was different in this experiment, but it did not make CP intake different since CP content and DM intake of all treatments were not significantly different. Intake of CP in this experiment ranging from 910 to 990 g/day exceeded protein requirement for cattle in tropical region with live weight of 200 kg and expected weight gain of 0.75 – 1.00 kg suggested by Kearl (1982), i.e., 622 – 690 g/day. According to Sudarman & Ito (2000) when crude protein offered higher than required, part of that will be converted as heat resulting in higher heat production. This will contribute to heat stress for the animal in hot environment. Thus providing cattle with higher protein diet than their requirement is not efficient in term of physiological and economical concern as the price of protein source feed is high. However, protein

Table 4. Nutrient intakes and digestibilities, body weight gain and feed efficiency utilization

Parameters	Treatment Diets*			
	RO	R1	R2	R3
DM Intake (kg/head/day)	6.01±0.39	5.84±0.18	5.73±1.06	5.62±0.96
CP intake (kg/head/day)	0.99±0.06	0.96±0.03	0.94± 0.18	0.91±0.16
CF intake (kg/head/day)	0.78±0.04	0.75±0.02	0.74±0.13	0.72±0.12
Daily gain (kg/head/day)	0.88±0.20	0.87±0.19	0.84±0.23	0.93±0.27
Feed utilization efficiency (%)	14.64±2.45	14.89±2.77	14.65±3.40	16.54± 2.76

*R0 = 90% concentrate + 0% coffee husk + 10% napier grass, R1 = 90% concentrate + 3.33% coffee husk + napier grass 6.67%, R2 = 90% concentrate + coffee husk 6.67 % + napier grass 3.33% and R3 = 90% concentrate + 10% coffee husk + napier grass 0%

Table 5. Nutrient digestibility (%) of experimental feed

Nutrients	Treatment Diets*			
	R0	R1	R2	R3
Dry matter	84.82±0,93	84.37±0.47	83.47±3.74	83.30±2.60
Crude protein	87.17±0.75	86.09±0.43	84.43±3.57	84.72±2.39
Crude fibre	69.51±1.67	67.89±0.85	67.40±7.07	65.83±5.21

*R0 = 90% concentrate + 0% coffee husk + 10% napier grass, R1 = 90% concentrate + 3.33% coffee husk + napier grass 6.67%, R2 = 90% concentrate + coffee husk 6.67 % + napier grass 3.33% and R3 = 90% concentrate + 10% coffee husk + napier grass 0%

Table 6. Income Over Feed Cost (IOFC) of Madura cattle fed ration with different fiber sources

Variables	Treatment Diets*			
	R0	R1	R2	R3
Total weight gain (kg/head) (1)	51.0	50.2	48.8	53.8
Cattle selling prices (Rp/kg/head) (2)	65,000	65,000	65,000	65,000
Gross Income (Rp/head) ((1)*(2)), (3)	3,315,000	3,263,000	3,172,000	3,497,000
Feed consumption (kg/head) (4)	348.5	338.9	332.4	326.2
Feed price (Rp/kg) (5)	3408	3337	3266	3194
Feed cost (RP/head) ((4)*(5)), (6)	1,187,879	1,130,936	1,085,607	1,041,595
IOFC (3-6)	2,127,121	2,132,064	2,086,393	2,455,405

*R0 = 90% concentrate + 0% coffee husk + 10% napier grass, R1 = 90% concentrate + 3.33% coffee husk + napier grass 6.67%, R2 = 90% concentrate + coffee husk 6.67 % + napier grass 3.33% and R3 = 90% concentrate + 10% coffee husk + napier grass 0%

utilization efficiency is not just based on the quantity, but also its quality.

Wiryawan et al. (2017) reported that feeding Madura cattle with complete ration consuming crude protein of 1300 and 800 g/head/day had daily gain of 674 and 570 g/head/day. These cattle daily gain is lower than those obtained in present experiment. Hence, what Kearl (1982) had suggested of the protein requirement for cattle in the tropics seem cannot be applied for all breed cattle and all kind of diets.

Eventhough the highest crude fibre content in present experiment was 13.04% in diet R3, however its crude fiber intake was not different from other treatments. Crude fiber content in this experiment was still in accordance with that of Luthfi et al. (2018) finding that the crude fibre content of ration for Madura cattle should be no more than 15.38%. Above this level will cause intolerant increase in methane production, reduce feed utilization efficiency and further decrease production performance (Luthfi et al. 2018). This is supported by Montenegro et al. (2016) and (Cottle et al. 2011) who reported that high crude fibre in the diet can cause high methane production which contribute to 8 – 14% lost of digestible energy ingested.

The treatment of feed with different fiber sources did not give a different effect (P> 0.05) on the feed utilization efficiency. This due to the data of dry matter intake and animal body weight gain, the factors that contribute to feed utilization efficiency, were also not significantly different. Feed efficiency utilization values in this study ranged from 14.59 - 16.36% (Table 4). Compared to the results of Rab et al. (2016) using Madura cattle with a value of feed utilization efficiency of 9.8%, the values obtained in this study were higher. Feed efficiency can be used as a criterion to determine the quality of feed given to livestock. High efficiency of feed used can be attributed to better nutrient content of the feed so that eventhough animals consume the same amount of feeds but produce better body weight.

Nutrient Digestibilities

Nutrient digestibilities of the diets used in the presnt experiment are shown in Table 5. Giving different sources of fiber in the ration had no different effect (P> 0.05) on dry matter digestibility. This is due to the composition of feed ingredients and consumption of dry matter from each treatment relatively similar. The digestibility of dry matter in this study ranged from 83.30 to 84.82% (Table 5). This digestibility values were comparable to the result of Wiryawan et al. (2017) by feeding Madura cattle with complete ration containing 15% and 30% soybean pod resulted in DM digestibility of 83 and 83.6%. Umar et al. (2007) reported that madura cattle fed a diet with concentrate and napier grass ratio of 46.5 : 53.5 with a crude fiber content of 21.36% resulted in a dry matter digestibility value of 65.51%. The higher digestibility of dry matter in this study than that of Umar et al. (2007) because the crude fiber content of feed in this study is lower which was arround 12.9% (Table 3). The higher crude fiber of a feed will cause the digestibility of the feed to be lower.

Crude protein digestibility in the study showed no significant difference (P> 0.05) between treatments. The absence of the influence of different fiber sources on crude protein digestibility because the quantity and quality of crude protein in all treatments are also relatively not different. This shows that feeding with different fiber sources at the level of up to 10% of experimental diet did not interfere the performance of rumen microbes in degrading crude protein.

The digestibility value of crude protein in this study were quite similar to those of the study reported by Wiryawan et al. (2017) with Madura cattle fed complete ration containing soybean pod of 15 and 30% with crude protein content of 15.2 and 15% resulted in crude protein digestibility of 89.4 and 87.0%, respectively, while in the present experiment the crude protein digestibility ranged from 84.43% - 87.17%. The crude protein content of the feed in this study was somewhat higher, i.e., 16.12 - 16.48% and TDN was rather low (Table 3) compared to those reported by Wiryawan et al. (2017). According to McDonald et al. (2011) protein digestibility of feed depends on protein quantity and quality in rations and rumen microbial activity. In line with that, Jayanegara et al. (2006) revealed that feeding with high crude protein content activates microbes in the rumen. The number of proteolytic bacteria in the rumen will be high followed by the deamination process which results in increased digestibility of feed organic matter. For rumen bacterial growth high protein supply should be provided with proper energy availability. In present experiment energy (TDN); protein ratio ranged from 3.98 – 4.07 and gave daily gain of 0.84 – 0.93 kg/day while the experiment (Wiryawan et al. 2017) energy (TDN) ; protein ratio were 4.49 and 4.55 and gave daily gain of 0.67 and 0.57 kg/day. Study on the different levels of energy and protein, Li et al. (2014) found that cattle given high energy diet combined with either low or high protein tended to result in higher gain than those fed low energy diet, i.e., 0.74 vs 0.64 kg/day.

Digestibility of crude fiber in this study also showed no significant difference (P> 0.05). This shows that feeding different fiber sources does not interfere with rumen microbial activity in degrading crude fiber. The digestibility value of crude fiber in the present study tends to be higher than that reported by Zulkarnaen (2017) using Bali cattle fed 30% concentrate and 70% fiber sources which yield crude fiber digestibility of 51.09%. Higher crude fiber digestibility value in this study was due to its crude fiber content of only 12.78 - 13.05% (Table 3) lower than that of Zulkarnaen (2017) which used rations with crude fiber content of 28.23%. It is a common knowledge that diet with higher crude fibre content usually has lower digestibility.

Income Over Feed Cost (IOFC)

Calculation results of Income Over Feed Cost (IOFC) are shown in Table 6. Giving different sources of fiber in feed statistically had no effect (P> 0.05) on IOFC values. This is because body weight gain and feed consumption in all treatments were also not significantly different.

However, numerically that is the important thing in business, the IOFC value of the R3 treatment was higher than those of the other treatments. This is because the price of coffee husk was cheaper than the prices of napier grass so that the cost of feed for R3 treatment becomes cheaper than those of the other treatments. Besides that body weight gain and feed utilization efficiency in R3 treatment were better than those of other treatments which contribute to the higher IOFC value of R3 treatment.

This study showed that 10% of napier grass (in dry matter) in beef cattle ration can be replaced by equal

portion of coffee husk dry matter. Indonesia produced 639,305 tons coffee cherry (Direktorat Jenderal Perkebunan 2016) and 20% of that is waste in form of coffee husk (Widyotomo 2013). Since dry matter content of coffe husk and napier grass are 86.91% and 14.17%, respectively, thus coffee husk can replace 784,220 tons fresh napier grass. This total amount of coffe husk can be used to feed 3,367,394 heads of beef cattle in 58 days finishing program with the ration of 90% concentrate and 10% coffee husk.

CONCLUSION

Coffee husk at 10% of the total feed can be used as a source of fiber to replace napier grass use during dry season without disrupting the performance of Madura cattle and producing higher IOFC values, thus giving higher profits to the cattle fattening business.

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Rhizopus oligosporus Activity in Crude Extract and Powder Form to Reduce *Aspergillus flavus* and Aflatoxin Contamination in Corn

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ABSTRAK

Kusumaningtyas E, Masrianti, Fitrya F. 2019**.** Aktivitas *Rhizopus oligosporus* dalam bentuk ekstrak kasar dan serbuk untuk menurunkan *Aspergillus flavus* dan aflatoksin pada jagung. JITV 24(4): 173-181. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2078

Rhizopus oligosporus (RO) dalam bentuk kultur isolat diketahui dapat menurunkan kontaminasi kapang toksigenik *Aspergillus flavus* (AF) dan aflatoksin B1 pada pakan ayam. Aplikasi dalam bentuk kultur kurang efektif. Penelitian ini bertujuan untuk mengevaluasi aktivitas *Rhizopus oligosporus* (RO) dalam bentuk ekstrak dan inokulum untuk menurunkan kontaminasi AF dan aflatoksin B1 pada jagung. RO dipanen dari kultur agar, sebagian diekstrak dengan cara dihancurkan, ditambah air dengan perbandingan 1:1 (w/v) dan disentrifus. Supernatant disaring menggunakan kertas saring Whatman 41. Inokulum dibuat dengan menginokulasikan RO pada tepung kedelai dan diinkubasi pada suhu 28^{o}C selama 5 hari. Inokulum dikeringkan pada suhu 40^{o}C selama 24-48 jam. Ekstrak dan inokulum masing–masing diuji aktivitasnya dengan menambahkankan pada jagung. Ekstrak diuji dengan perbandingan ekstrak dan jagung 1:1 (v/w), sedangkan inokulum ditambahkan pada jagung dengan dosis 5, 25, 50, 100 dan 200 g/kg jagung. Pengujian kadar aflatoksin B1 dilakukan menggunakan kit ELISA aflatoksin. Hasil penelitian menunjukkan bahwa ekstrak RO dapat menurunkan kontaminasi AF hingga 1 log 10, sedangkan konsentrasi inokulum terendah yang dapat menghambat pertumbuhan AF hingga 6 log 10 adalah 100 g inokulum/kg jagung. Ekstrak RO 125 dan 250 mL/kg jagung dapat menurunkan kontaminasi aflatoksin sebesar 93,69% dan 85,84%. Inokulum pada dosis 5 dan 100 g/kg jagung dapat menurunkan konsentrasi aflatoksin sebesar 57,58% dan 85,94%. Berdasarkan hasil tersebut dapat disimpulkan bahwa RO dalam bentuk ekstrak maupun inokulum dapat menurunkan kontaminasi AF dan aflatoksin B1 pada jagung. Rhizopus dalam bentuk inokulum lebih mudah diaplikasikan daripada dalam bentuk ekstrak.

Kata Kunci: Aflatoksin, *Aspergillus flavus*, Ekstrak, Serbuk, *Rhizopus oligosporus*

ABSTRACT

Kusumaningtyas E, Masrianti, Fitrya F. 2019**.** *Rhizopus oligosporus* activity in crude extract and powder form to reduce *Aspergillus flavus* and aflatoxin in corn. JITV 24(4): 173-181. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2078

Rhizopus oligosporus (RO) in isolate culture was known to reduce contamination toxigenic mold *Aspergillus flavus* (AF) and aflatoxin B1 in chicken feed. Application in culture form was not effective. The aim of this research was to evaluate RO activities in extract and inoculum form to reduce contamination of AF and aflatoxin B1 in corn. RO was harvested from agar plate, blended, added with water (ratio 1:1 (w/v)) and centrifuged. Supernatant was filtered using Whatman 41. Inoculum was made by inoculation RO in soy powder and incubated at 28^{o}C for 5 days. Inoculum was dried at 40^{o}C for 24-28 hours. Assay was conducted by addition extract or inoculum to corn. Extract and corn ratio were 1:1 (v/w), while inoculum doses were 5, 25, 50, 1000 dan 200 g/kg corn. Assay for aflatoxin B1 was done using kit ELISA aflatoxin. The result of this research showed that extract was able to reduce AF contamination up to 1 log 10, while the less concentration of inoculum which able to inhibit AF up to 6 log 10 was 100 g/kg corn. Extract RO 125 and 250 mL/kg corn was able to reduce aflatoxin contamination by 93.69 % and 85.84 %. Inoculum at dose 5 and 100 g/kg corn was able to reduce aflatoxin 57.58% and 85%. Based on the result, it could be concluded that RO in extract or inoculum form was able to reduce contamination of AF and aflatoxin B1 in corn. *Rhizopus* as inoculum was easier to be applied than in extract form.

Key Words: Aflatoxin, *Aspergillus flavus*, Extract, Powder, *Rhizopus oligosporus*

INTRODUCTION

Aspergillus flavus is a toxigenic mold producing aflatoxin. This mold commonly grows in seeds and nuts. Aflatoxin is a carcinogenic substance which harmful to human health and cause reduction in animal production. Some technologies were applied to minimize aflatoxin exposure during pre and post-harvest such as irradiation, ozone fumigation, chemical and biological control agent and improving packaging

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materials (Udomkun et al. 2017). However, these technologies may not sufficient to eliminate the fungal and toxin contamination (Ortega-Beltran et al. 2018), therefore it need to try new methods or agents. Bacteria and non-toxigenic fungi were commonly used for *Aspergillus flavus* biocontrol and aflatoxin degradation. *Streptomyces yanglinensis* 3-10 (Shakeel et al. 2018) and *Bacillus subtilis* UTBSP1 (Farzaneh et al. 2016) showed promising activity to control *Aspergillus flavus* in peanut kernel and pistachio nut. Non-toxigenic *Aspergillus flavus* (Ehrlich 2014) and *Rhizopus stolonifer* (Iqbal et al. 2014) as mycelium or extract inhibited *Aspergillus flavus* growth. Non-toxigenic fungus competes with close related strain to meet nitrogen, carbon, water and mineral (Ehrlich 2014). *Rhizopus oligosporus* (BCC F0216), *Saccharomyces cerevisiae* (BCC F0206) (Kusumaningtyas et al. 2006), *Rhizopus oryzae* (CCT 7560) and *Trichoderma reesei* (QM9414) (Hackbart et al. 2014) were also reported as biological control for *Aspergillus flavus* or aflatoxin.

Direct application of *Rhizopus oligosporus* in chicken feed was reported able to inhibit growth of *Aspergillus flavus* and reduce aflatoxin production up to 15 days (Kusumaningtyas et al. 2006). Addition of *Rhizopus oligosporus* in duck feed reduced aflatoxin B1 and M1 residue in duck liver (Kusumaningtyas et al. 2006b). Direct application of *Rhizopus oligosporus* culture from agar plate was not effective, especially in field. Mixing *Rhizopus oligosporus* in rice powder was reported able to maintain viability of *Rhizopus* 100% in 60 days (Kusumaningtyas et al. 2005). In this research was tried to used soy powder to replace rice powder. Soybean is not only rich in protein, but also contains almost all essential and non-essential amino acids. Besides, soybean less expensive than animal protein. Fermentation increases protein and fats content and decreases carbohydrates, increase small size peptides (<15 kDa), improved amino acids essential and reduced anti-nutritional substance in soy (Mukherjee et al. 2016). Inoculum was made by growing *Rhizopus oligosporus* in soy powder. The dry inoculum was applied in corn to reduce aflatoxin contamination. The result was compared to *Rhizopus oligosporus* extract. This study was done to evaluate *Rhizopus oligosporus* in extract and inoculum form to reduce *Aspergillus flavus* and aflatoxin contamination in corn.

MATERIALS AND METHODS

Microorganism

Rhizopus oligosporus (BCC F0216) and *Aspergillus flavus* (BCC F0213) were obtained from BBlitvet Culture Collection.

Culturing and propagating microorganism

Rhizopus oligosporus (BCC F0216) and *Aspergillus flavus* (BCC F0213) were grown in Sabouraud dextrose agar (SDA), incubated at 28ºC for 3 days. Fresh cultures were then used directly for assays.

Extraction of *Rhizopus oligosporus*

Rhizopus oligosporus (RO) from Sabouraud dextrose agar (SDA) media was harvested by scrapping mold, put into new flask and suspended with 7.5 mL phosphate buffer saline (PBS) per petri dish. Suspension was blend, homogenized and then centrifuged at 6000 xg for 15 minutes. Supernatant was taken and then filtered through 0.45 µm membrane. Filtrat was collected in the new tube. Filtrat was applied diretly for assay or storage at -20ºC until used. Filtrat might contain secondary metabolite, protein, enzyme and other soluble compound.

Soybean powder preparation

Dry soybean was ground until it become flour. The flour was sterilized by autoclaving at 121ºC, 1 atm for 20 minutes (WHO 2019).

Rhizopus oligosporus Inoculum

Rhizopus oligosporus (RO) was grown on the SDA media and incubated at 28ºC for 3 days. RO was harvested and suspended in PBS until reach 10^6 spores/mL. As much 250 mL spore suspension was added into 500 g sterile soybean flour, homogenized and then incubated at 28ºC for 3 days. Inoculum was stirred every day to ensure homogeneity. After 3 days incubation, inoculum was divided into two part. Once was without treatment, namely wet inoculum, and used for assay directly. Another part was dried at 40ºC overnight or until dried, namely dried inoculum. Killed inoculum was made by autoclaved the inoculum at 1 atm, 121ºC for 20 minutes.

Corn contaminated by *Aspergillus flavus*

Ground corn was sterilized by autoclaving at 121ºC, 1 atm for 20 minutes. *Aspergillus flavus* (AF) was prepared by growing in SDA medium, incubated at 28ºC for 3 days. Spores were collected by scraping and suspended in sterile PBS. The suspension was diluted to obtained spore concentration 10^6 spores/mL. As much 10 mL AF spore suspension was added into 250 g sterile corn, homogenized and then incubated at 28ºC for 3 days.

Effect of *Rhizopus oligosporus* extract on growth of *Aspergillus flavus* in corn

RO extract was mixed with corn contaminated by *A. flavus* in various concentrations: 10^6, 10^5, 10^4, 10^3, 10^2 CFU/g with ratio 1:1 (v/w), incubated at room temperature for 2 hours. The mixture was taken 1 gram diluted with physiological NaCl until the volume reached 10 mL. Serial dilution was made from 10^{-1} to 10^{-6}. The 1 mL suspension was poured in SDA medium (±50ºC) and let the medium hardened. The plates were incubated at 28ºC for 3 days (Ahmed et al. 2016) . The colony grown was counted and recorded.

Effect of killed inoculum of *Rhizopus oligosporus* in *Aspergillus flavus* growth

As much as 100 g killed inoculum was added to the 1 kg corn contaminated with AF which was made previously, incubated in room temperature for 2 hours. As much as 1 g of the mixture was taken and diluted with physiological NaCl until the volume reached 10 mL. Serial dilution was made from 10^{-1} to 10^{-6}. The 1 mL suspension was poured in SDA medium (±50ºC) and let the medium hardened. The plates were incubated at 28ºC for 3 days. The colonies grown were counted and documented.

Effect of wet and dried of live inoculum activity in *Aspergillus flavus* growth

Each of wet and dried inoculum 100 g was added to 1 kg corn contaminated with AF and incubated at 28ºC for 3 days. As much as 1 g of each mixture was taken and diluted with physiological NaCl until the volume reached 10 mL. Serial dilution was made from 10^{-1} to 10^{-6}. The 1 mL suspension was poured in SDA medium (±50ºC) and let the medium hardened. The plates were incubated at 28ºC for 3 days. The viable colonies were counted and documented. Corn contaminated with AF without treatment was used as control.

Effect of concentration of dried *Rhizopus* inoculum on *Aspergillus flavus* reduction in corn

RO inoculum was prepared in sterile flask containing: 5 g, 25 g, 50 g, 100 g, 50 g and 200 g. Each inoculum was mixed with 1 kg corn contaminated which previously contaminated by *Aspergillus flavus* (AF). Corn contaminated (AF) without inoculum addition was used as negative control. All treatments were done in three replications. Each mixture was incubated at 28ºC for 3 days. After 3 days incubation, 1 g mixture was taken and suspended in 9 mL physiological NaCl. Serial dilution was made from 10^{-1} to 10^{-6}. The 1 mL suspension was taken and inoculated in SDA medium using pour plate methods. The plates were incubated at 28ºC for 3 days. The colony grown was counted and documented.

Effect of inoculum on growth of *Aspergillus flavus* during certain period

As much as 100 g RO inoculum was mixed with 1000 g corn contaminated with *A flavus.* One g of the mixture was taken at day 0, 1, 2, 3, 4, 5, 6, 7, 8. Corn contaminated with *A. favus* without inoculum addition was used as negative control. The 1 g of the mixture was suspended in 9 mL physiological NaCl. Serial dilution was made from 10^{-1} to 10^{-6}. The 1 mL suspension was inoculated in SDA medium using pour plate methods. The plates were incubated at 28ºC for 3 days. The colony grown was counted and documented.

ELISA for Aflatoxin

Aflatoxin extraction was conducted by taken 25 g corn treated previously, added with 100 mL ethanol 70%, shaken for 30 minutes and then filtered. Filtrat was transferred in new tube. Aflatoxin ELISA kit (BBLitvet) was conditioned at room temperature for 15-30 minutes. Aflatoxin B1 standard 40 µL with concentration: 0, 0.12, 0.37, 1.1, 3.3, 10,30 ppb and extract: negative control (corn treated with A flavus without RO inoculum), sample 1 (corn treated with RO extract with ratio 1:1 (g/mL), sample 2 (corn with inoculum 50 g/kg corn) and sample 3 (corn with inoculum 200 g/kg) was put in the microplate. Each plate was added with 120 µL conjugate AFB1-HRP and mixed well. The mixture was taken 60 µL for each microplate well and move to new microplate coated with antibody to aflatoxin B1. The plate was shaken to ensure all mixed well. Plate was incubated room temperature for 30 minutes. All suspension were removed and washed 3 times. Tetramethylbenzidine was added to each well and let it around 15 minutes until form blue colour. Stop solution was added to stop reaction until form yellow color. The plate was read at wavelength 450 nm in microplate reader. Aflatoxin concentration was calculated based on standard curve. Percentage of inhibition was calculated based on:

$$\mathbf{y = a \ln X + b}$$

y= % inhibition of sample; a= a slope; b= intercept;
X= sample concentration

RESULTS AND DISCUSSION

Rhizopus oligosporus was added to corn as extract and live inoculum in wet and dried form. Wet and dried inoculum were assayed for viability before used. It showed that they still viable even after drying process.

Effect of *Rhizopus oligosporus* (RO) extract on growth of *Aspergillus flavus* (AF) in corn

RO extract was added to ground corn in different concentration of AF contamination. This treatment was performed to determine the effectivity of the extract to decrease of *A. flavus* contamination. As shown at Figure 1, RO extract worked well in maximum AF concentration 10^3 CFU/g with reducing value around 1 log cycle. When live culture of RO was added to chicken feed contaminated by AF, it was able to inhibit AF growth by competition mechanism (Kusumaningtyas et al. 2006). RO grew faster and compete to take mineral source with AF therefore Af was inhibited. In this research, the use of extract was proposed to evaluate that was only competition or RO produce certain metabolites which able to inhibit growth of AF.

As shown in Figure 1, AF inhibition was not only caused by competition but also metabolite produced by RO. Extract did not contain RO micelia due to membrane filtration but the extract still able to reduce AF, especially when AF in $\leq 10^3$ CFU/g. The extract might contain celuler or extracellular metabolite which able to inhibit AF. Although RO metabolite has not been reported, Lanciotti & Guerzoni (1993) was revealed that the similar genus *Rhizopus arrhizus* produced metabolites such as ethanol, isobutilalcohol and 3-metil butanol which was able to inhibit AF growth. *Rhizopus oryzae* produce secondary metabolite methyl eugenol which showed inhibition of AF colonization and aflatoxin production on peanut and kernel (Faisal & Prasad 2016). Kobayasi et al. (1992) reported that RO produced protease which cut proteins into peptides and also inhibited AF growth.

RO extract showed good in AF growth inhibition in AF concentration $\leq 10^3$ CFU/g (Figure 1). In concentration $\geq 10^4$ CFU/mL, the ability of RO extract to inhibit AF growth much decreased might be the metabolite did not enough to kill in higher concentration. Decreased activity could occur due to metabolite concentration constant but AF concentration increased or the metabolite decreased and AF constant. Similar case was shown by antifungal activity of secondary metabolite of *Trichoderma koningii* IABT 1252'S. Antifungal activity of the metabolite decreased simultaneously with reduction of metabolite concentration (Rabinal & Bhat 2017).

Comparison of extract and killed RO inoculum activity in *Aspergillus flavus* growth

The metabolite released by RO assumed that was able to stable although the RO was killed or it mass was removed. This may the metabolite present both in extract and killed inoculum. Comparison of extract or killed inoculum activity in AF growth was described in the Figure 2.

The activities of extract and killed RO inoculum to inhibit AF growth seem not different. Their inhibition less than one log cycle that means extract or killed inoculum only decrease AF from 10^6 CFU/g to 10^5 CFU/g. It means that the extract or inoculum activity to inhibit AF growth was low. As mentioned previously, RO extract very low activity to inhibit AF in AF concentration more than 10^3 CFU/g. The important substance might be released to extracellular but degraded during extraction process. Blending process might destruct the active compound. For example, the active substance which expected able to degrade aflatoxin was inactivated by intracellular enzyme. Intracellular enzyme was released during crushing and blending process. In addition, Filtration might cause active substance trapped in the filter and did not present in the filtrate. In killed inoculum, the substance might be degraded due to high temperature and pressure.

Comparison of wet and dried of live inoculum activity in *Aspergillus flavus* growth

Rhizopus oligosporus (RO) extract, wet and dried inoculum were prepared for further assay (Figure 3a and 3b). Dried inoculum in powder form easier to be mixed with corn than wet inoculum. To evaluate that live inoculum was able to inhibit AF growth was conducted by assayed wet and dried inoculum. Drying process was performed to simplify application and did by oven drying at 40^o C to avoid substance disruption.

As shown at Figure 4, there is no different result between wet and dried inoculum to inhibit AF. They were able to reduce *Aspergillus flavus* (AF) up to 6 log cycle CFU/mL. This proves that both wet and dried inoculum were able to be applied to reduce AF in ground corn. Comparison this result with previously performance in extract and killed inoculum showed that the active substance to inhibit AF was present in live *Rhizopus.* The substance might be damage when *Rhizopus* was extracted or killed. Nout (1989) reported that *Rhizopus* and *Neurospora spp* which inoculated simultaneously in mashed groundnuts were able to inhibit growth of *Aspergillus flavus*. *Aspergillus spp* were able to grow but lesser extend and visually different mycelial development and sporulation behavior.

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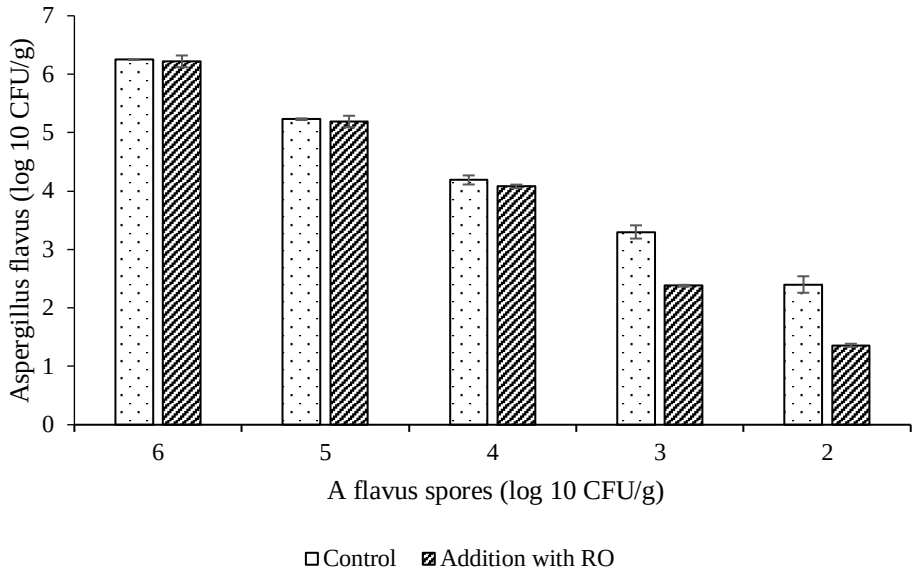

Figure 1. The ability of *Rhizopus oligosporus* extract to reduce*Aspergillus flavus* contamination in corn

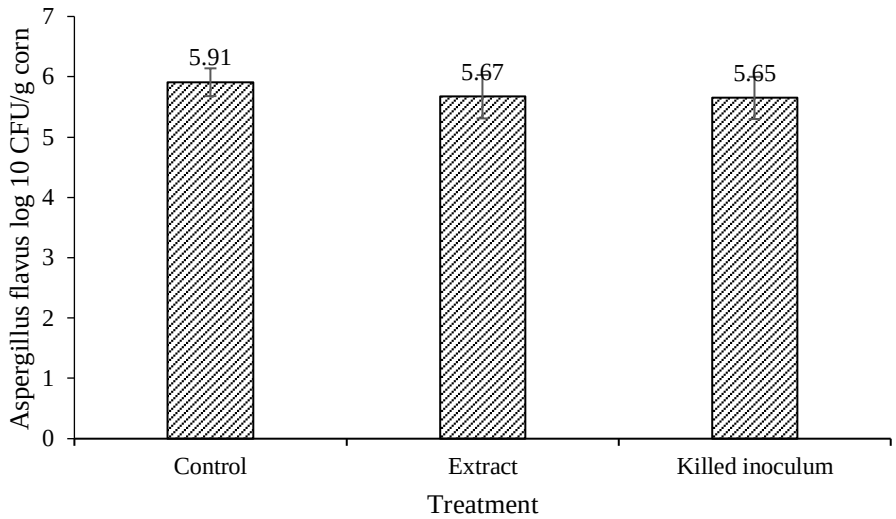

Figure 2. Comparison of the activities of extract and killed inoculum activities to inhibit *Aspergillu flavus*

a b

Figure 3. Wet (a) and dried (b) inoculum of *Rhizopus oligosporus*

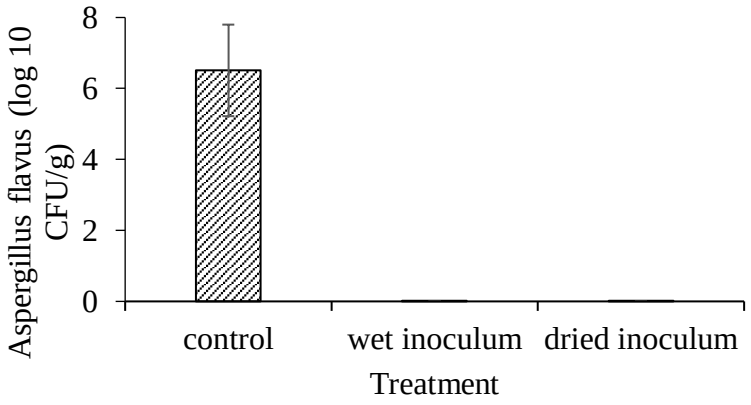

Figure 4. Comparison of the activities of wet and dried inoculum to inhibit *Aspergillus flavus*

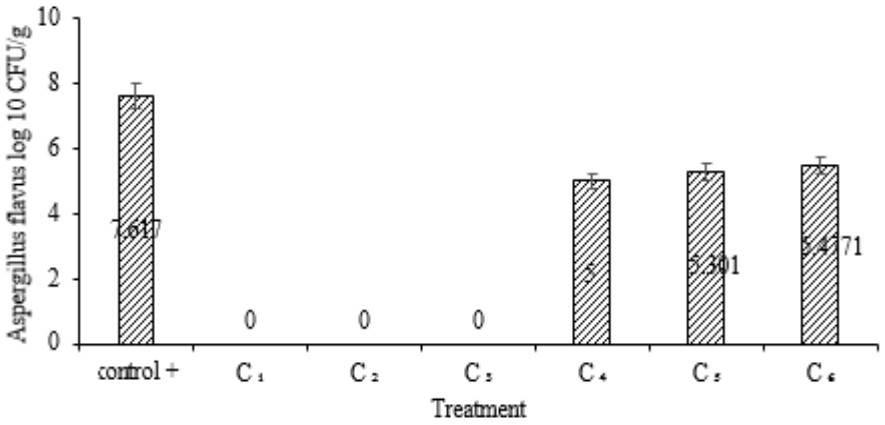

Figure 5. The effect of dried *Rhizopus oligosporus* inoculum in various concentration. Control: 1 kg corn contaminated *Aspergillus flavus* without any treatment and in the treatment the contaminated corn were added with dried inoculum C1: 200g, C2: 150 g, C3: 100 g, C4: 50 g , C5: 25 g and C6: 5 g

Rhizopus produces metabolite such as C compound which inhibit *Aspergillus* growth. Previously, although the Rhizopus extract was also from live *Rhizopus*, extraction process inactivated active compound. It is also possible that the antifungal substance which active to inhibit AF, present in RO mycelia. Fungal secondary metabolites produced and released from asexual conidia and sexual fruiting body structure (Doll et al. 2013). Antifungal substance in dried inoculum was stable and not degraded in 40ºC oven drying. Dried inoculum was also more applicable in field due to its form. Powder form was easier to be mixed with food or feed stuff like corn or chicken feed than wet form.

The effect of concentration of dried RO inoculum on AF reduction in corn

Dried *Rhizopus* inoculum in various concentrations was applied in ground corn. It was used 200g, 150g, 100g, 50 g and 5 g per kg corn. The result was shown in Figure 5. The inoculum performed well in concentration 200, 150 and 100 g/kg corn indicated by reduction of *Aspergillus flavus* (AF) from 7.617 log 10 CFU/g to 0. In dose 50g/kg reduction only around 2 log cycle. However, reduction AF activity among dose 50g, 25g and 5 g did not much different. The reduction decreased along with reduction of inoculum dose. Therefore, application of inoculum to reduce AF contamination is dose dependent. In addition, minimal dose which applicable in field was not less than 100 g/kg to obtained sufficient result.

This result need further research to apply the inoculum in field. Preliminary study done previously showed that application inoculum in powder form producing better result than granule. Powder form mixed better in chicken feed. Granule form tend to settle in the bottom of the flask. Small size particle of powder made it easier to interact to food or feed surface contaminated by AF.

Critical point in the application of RO inoculum was mixing process beside the dosage. Amount of corn which would be added with inoculum determined the

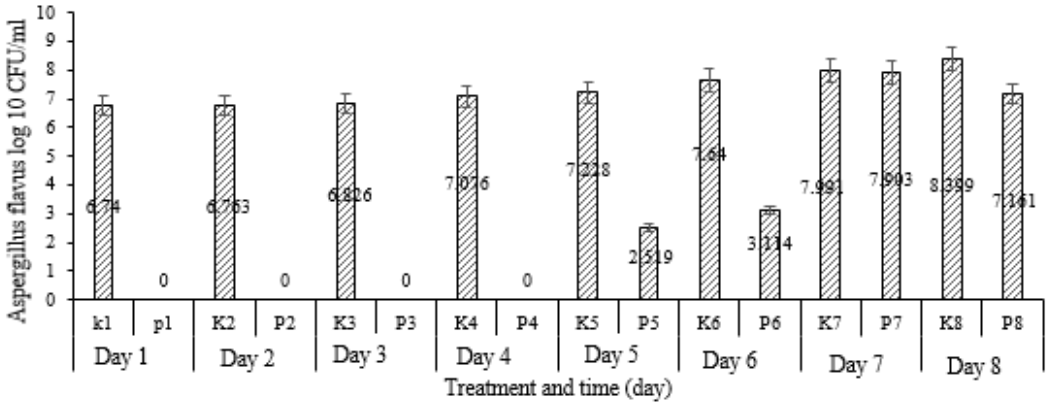

Figure 6. The effect of dried *Rhizopus oligosporus* inoculum 100 g/kg contaminated corn in 8 days observation

quality of mixing process and reducing AF contamination. For example, mixing in 1 kg corn was easier than 100 kg corn and it increase reducing AF activity.

The effect of RO inoculum to reduce AF certain period of time

Dried inoculum dose 100g/kg was the lowest concentration resulting sufficient result. The following treatment was application of RO inoculum in ground corn with dose 100g/kg and stored for 8 days. AF reduction was observed and evaluated every day. The inoculum reduced AF 100% in 4 days incubation. At day 5 AF grew around 2 log 10 CFU/g and tend to increase every day. Based on this data, corn or other food or feed stuff contaminated with AF around 10^7 CFU/g and treated with RO inoculum better consumed before 5 days. Food and feed contaminated AF at previous dose without RO inoculum do not allowed for consumption both by animal and human. The effect of dried RO inoculum 100 g/kg contaminated corn in 8 days observation was shown in Figure. 6.

Reduction aflatoxin contamination in corn by extract and dried RO inoculum

The FDA guidelines based on maintaining performance and avoiding disease related to aflatoxin mentioned that aflatoxin level in corn for young animal and dairy cattle must less than 20 ppb, corn for breeding beef cattle swine and mature poultry less than 100 ppb, corn for finishing swine and finishing cattle less than 200 and 300 ppb (FDA 2016).

Specific microbial metabolites showed strong activities to inhibit aflatoxin production. A number of *Lentinula edodes* isolates was able to inhibit aflatoxin production. This is possible that different compound needs different strategies to obtain optimum activity. A number of metabolites or filtrates increase their activities after fractionation, but fractionation of *L edodes* CF42 filtrates decrease their inhibitory effect (Tian & Chun 2017).

RO extract, wet and dried inoculums were able to reduce and inhibit growth of AF in corn. In the following step was measurement aflatoxin B1 as a secondary metabolite of AF with or without treatment with extract and dried *Rhizopus* inoculum. Aflatoxin B1 concentration measurement was conducted using ELISA methods and the results were shown in Table 1.

As shown in the Table 1, *Rhizopus oligosporus* (RO) both extract and dried inoculum were able to reduce aflatoxin contamination in corn. Reduction aflatoxin production might be due to inhibition of *Aspergillus flavus* (AF) growth which cause decreasing aflatoxin production. It is also possible that RO metabolites bound and degraded aflatoxin resulting reduction of measured aflatoxin. Although RO in extract form only inhibit AF less than 1 log cycle in ratio 1:1 (v/w) (Fig 3.) but it was able to reduce aflatoxin up to 93.69 % in ratio 1:4 (v/w) (Table 1). This might the RO soluble metabolites in extract less activity to inhibit AF growth but active to reduce aflatoxin contamination in corn. Consider that incubation time for extract to react with AF and aflatoxin for 2 hours, it is possible that the RO metabolites was act as toxin binder or degraded aflatoxin which already present in corn resulting reduction in aflatoxin measurement.

Dried inoculum contained life RO which may also grow and compete with AF cause AF inhibition and aflatoxin reduction. Other species, *Rhizopus nigricans* which coculture with aflatoxin producer fungi, *Aspergillus parasiticus* was reported to inhibit aflatoxin production (El-Shiekh et al. 2007). Nout (1989) also

Table 1. Reduction of Aflatoxin B1 concentration in corn treated with *Rhizopus oligosporus* extract and dried inoculum

No	Treatment	Aflatoxin B1 (ppb)	Reducing Aflatoxin B1 (%)
1	Control contaminated corn without treatment	30.44	-
2	250 mL *Rhizopus oligosporus* extract/ kg contaminated corn	1.92	93.69
3	125 mL *Rhizopus oligosporus* extract/ kg contaminated corn	4.31	85.84
4	100 g *Rhizopus oligosporus* inoculum/ kg contaminated corn	4.28	85.93
5	50 g *Rhizopus oligosporus* inoculum/ kg contaminated corn	12.91	5759

revealed that *Rhizopus* and *Neurospora spp* produce metabolite C compound which inhibited *Aspergillus flavus*, aflatoxin production and degraded aflatoxin Other possible mechanism was the live RO also produce metabolites which bound and degraded aflatoxin beside AF growth inhibition. Consider that aflatoxin is toxic metabolite of AF, reduction of aflatoxin contamination was very important. Although RO extract also reduce aflatoxin but more difficult to be applied in field due to its liquid form, therefore dried inoculum is the most suitable form for RO application in food and feed stuff.

CONCLUSION

Rhizopus oligosporus in crude extract, wet inoculum and dried inoculum were able to inhibit *Aspergillus flavus* growth. Wet and dried inoculum inhibit *Aspergillus flavus* growth better than extract. Wet and dried inoculum had similar activity to inhibit *Aspergillus flavus* growth but the dried (powder) form easier to be mixed and applied in food or feed stuff. Extract and powder form of RO were also able to reduce aflatoxin B1 contamination in corn. The dried or powder form of RO was the most suitable form for application RO in field.

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Supplementation of Black Soldier Fly (*Hermetia illucens*) on Activity and Capacity Phagocytic Macrophage of Laying Hens

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ABSTRAK

Irawan AC, Rahmawati N, Astuti DA, Wibawan IWT. 2019. Suplementasi black soldier fly (*Hermetia illucens*) untuk aktivitas dan kapasitas makrofag fagositosis pada ayam petelur. JITV 24(4): 182-187. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2025

Larva *Black Soldier Fly* bersifat sebagai antibiotik alami. Pemanfaatan larva BSF dalam ransum unggas sebagai alernatif sumber protein konvensional, diharapkan dapat meningkatkan status kekebalan dan menjaga kesehatan ternak sehingga mengurangi penggunaan *antibiotic growth promoters* (AGP). Penelitian ini bertujuan mengevaluasi pengaruh jenis pemberian protein BSF terbaik untuk mengetahui status kesehatan ayam petelur berdasarkan aktivitas dan kapasitas fagositosis makrofag terhadap *Staphyllococcus aureus* non protein A. Hasil penelitian ini menunjukkan bahwa perlakuan BSF ekstrak (P3) memiliki nilai kapasitas fagositosis tertinggi, hal ini membuktikan BSF ekstrak dapat memicu sel makrofag bekerja maksimal memfagosit sel bakteri atau partikel asing. Rataan nilai aktivitas dan kapasitas fagositosis makrofag peritoneum menunjukkan perlakuan BSF ekstrak (P3) tertinggi, masing – masing sebesar 91.34 ± 0.38% dan 22.84 bakteri makrofag^{-1}.

Kata Kunci: Aktivitas dan Kapasitas Fagositosis Makrofag Peritoneum, *Antibiotic Growth Promoters* (AGP), *Black Soldier Fly* (BSF)

ABSTRACT

Irawan AC, Rahmawati N, Astuti DA, Wibawan IWT. 2019. Supplementation of black soldier fly (*Hermetia illucens*) on activity and capacity phagocytic macrophage of laying hens. JITV 24(4): 181-187. DOI: http://dx.doi.org/10.14334/jitv.v24i4.2025

Black Soldier Fly are natural antibiotics. It is expected that the use of BSF larvae in poultry rations as an alternative source of conventional protein will contribute to improving the immune status and maintaining animal health, thereby reducing the use of antibiotic growth promoters (AGPs). This study aimed to evaluate the effect of the best type of BSF protein for determining the health status of laying hens based on the activity and capacity of macrophage phagocytosis on the non-protein A bacterium *Staphyllococcus aureus*. Results of this study indicated that the BSF extract (P3) has the highest phagocytic capacity value. This result proves that the BSF extract can induce macrophage cells to optimally process bacterial cells or foreign phagocyte particles. The highest average activity value, and phagocytic capacity of peritoneal macrophages was resulted from BSF extract (P3), respectively was 91.34 ± 0.38% and 22.84 macrophage^{-1} bacteria.

Key Words: Activity and Capacity Of Phagocytic Peritoneal Macrophages, Antibiotic Growth Promoters (AGPs), Black Soldier Fly (BSF)

INTRODUCTION

The role of AGPs (antibiotic growth promoters) can increase chicken growth by 4-8% and feed conversion by 2-5% and kill pathogenic bacteria in chicken digestion track, such as, Salmonella sp., Campylobacter sp., Enterococci sp. and Escherichia coli. Negative effects of using antibiotics that are uncontrolled and inappropriate may lead to microbial resistance to antibiotics. The use of AGP increases the resistance or residues in the body, although it helps to increase chicken performance. Meeting protein needs in animal feed through the substitution of conventional food ingredients is the research focus. Fishmeal in Indonesia still depends on imports, but the price is expensive causing poultry farmers are having difficulty to maintain the business. Alternative solutions for animal protein needed at affordable prices and as a replacement for AGP, is black soldier fly (*Hermetia illucens*).

Insects have advantages over casein, insects boost immunity because they contain defensins, which are components of protein that is rich in cystine (Lv et al. 2016). Insects containing amino acid-derived antibacterial peptides can be used as antibacterial agents by Ravi et al. (2011), the fat content of an insect-assisted insect peptide in the body will activate lysozyme enzymes so that it can increase immunity and active cell phagocytosis (Villarroel et al. 2010).

The nutrient content of BSF (*Hermetia illucens*) includes: energy 5,282 Kcal GE / kg, crude protein 42,1%, fat 26%, calcium 7,56% and phosphorus 0.9% (St-Hilaire et al. 2007: Makkar et al. 2014). It is reported that the calcium mineral contained in the BSF flour was 88% (Finke 2013). According to Rambet et al. (2016) it is possible to replace fishmeal with BSF larvae flour up to 100% for fattening diet, with the digestibility of dry matter (57.96 – 60.42%), energy (62.03 – 64.77%) and protein (59-75. 32%). The BSF larvae has a protein content of 40-50% and a fat content of 29-32% (Bosch et al. 2014).

Hermetia illucens also have protease, amylase and lipase enzymes. Proteases convert proteins into amino acids, amylases convert starch into maltose and lipases convert fats into fatty acids and glycerol (Kim et al. 2011). BSF larvae are natural antibiotics. It has been reported that BSF larvae extracted with methanol solvent showed antibiotic properties in Gram-negative bacteria such as *Klebsiella pneumonia*, *Neisseria gonorrhoeae* and *Shigella sonnei*. Conversely, the results of the analysis also showed that larval extract was not effective for Gram-positive bacteria such as *Bacillus subtilis*, *Streptococcus mutans* and *Sarcina lutea* (Choi et al. 2012). According to Kim & Rhee (2016), high-lauric BSF larvae are a type of fatty acid that acts as a natural antimicrobial agent and chitin. BSF larvae were able to reduce *Salmonella enterica* serovar *Enteritidis* populations in human feces up to 6 $\log^{10}$ for 8 days, but were not effective for *Enterococcus* sp. and X174 bacteriophages (Lalander et al. 2013). These BSF larvae were able to reduce the population of *Escherichia coli* O157: H7 and *Salmonella enterica serovar enteritidis* in poultry feces (Erickson et al. 2004) and *E. coli* in dairy cows (Liu et al. 2008).

MATERIALS AND METHODS

The larvae of BSF were obtained from PT. Biocycle Indo Bogor. The larvae used were fed rations in the form of crude palm oil and harvested at the age of 15 days. After harvested, the larvae were treated in 3 forms, namely fresh BSF, dried BSF and BSF extract.

Fresh/life BSF

The method used for this treatment was self-mixing system, according to the nutrient requirements of the laying hens. The feed ingredients consisted of corn, rice bran, coconut oil, fish meal, soybean meal, $CaCO_3$, salt, premix, methionine and lysine were evenly mixed in the base ration for the control treatment, while for the fishmeal was reduced into 5%, and fresh BSF was given upto 8%. Fresh BSF was administered at top of basal diet (topping) in the tray.

Dried BSF

The first stage of dried BSF production was to separate pupa from the cocoon layer by washing and steaming at 90°C for 10 minutes. The larvae were then heated to 55°C for 24 hours to remove the water. Then the larva was ground to flour with a blender and put in airtight plastic. The percentage of fishmeal used in the basic ration was reduced to 5% and 8% BSF dried. The drying process of BSF may increase the possibility of transmitting pathogenic bacteria to livestock such as *Salmonella sp.* (Lalander et al. 2013).

Extract BSF

The steps for producing the BSF extract were carried out according to the modified method of Choi et al. (2012). The BSF larvae were washed and steamed for 10 minutes at 90°C, then the larvae were heated at 55°C for 24 hours to remove water, then the larvae were ground to flour with a blender and then with a denanol ratio of 1:10 (w/v) for 24 hours at room temperature, 2x filtered solution using whatman paper then use a reduced pressure rotary evaporator at 40ºC to get BSF methanol extract.

Maintenance of laying hens

Laying hens were kept in group battery cages (4 treatments x 5 replications x 10 laying hens). Maintenance was carried out for 8 weeks, starting with the age of 18 weeks to 26 weeks. Provision of 120 g / head / day ration and drinking water was given in ad libitum. Provision of treatment rations was given twice a day at 08.00 am and at 03.00 pm.

Activity and capacity of phagocytic peritoneal macrophages test

This test was performed at the end of the study using 20 laying hens from 4 treatment rations, each consisting of 5 repetitions. In the first step, all hens were injected intraperitoneally with a dose of 1 ml of *Staphylococcus aureus* non protein A at a dose of 1 ml containing 10^5 CFU / ml of bacterial particles for 4 hours. In the next phase, laying hens were slaughtered, stretched and then cuts were made on both strips.

Table 1. The nutritional content of laying hens feed during the study

Ingredients	Treatment (%)			
	P0	P1	P2	P3
Maize	56.12	54.67	54.50	54.01
Bran	7.01	3.49	5.41	6.23
Coconut oil	1.98	1.95	1.70	1.96
Soybean meal	22.65	22.45	20.84	19.86
Fish meal	08.00	5.00	5.00	5.00
Fresh BSF	-	8.00	-	-
Dried BSF	-	-	8.00	-
Extract BSF	-	-	-	8.00
$CaCO_3$	3.49	3.66	3.61	3.70
Salt	0.24	0.26	0.22	0.42
Premix	0.20	0.23	0.36	0.42
Methionine	0.20	0.12	0.16	0.21
Lysin	0.10	0.17	0.20	0.21
Total	100	100	100	100
Proximate composition[1)]				
Dry Matter (%)				
Crude Protein (%)	20.16	20.01	20.00	20.00
Crude Fat (%)	5.31	6.70	6.96	6.63
Crude Fiber (%)	2.78	2.92	3.20	3.34
Metabolis Energy (kkal kg^{-1})	2900.55	2908.10	2901.43	2903.88
Calsium (%)	4.00	4.01	4.00	4.10
Phosphor (%)	0.36	0.27	0.27	0,27
Lysin (%)	0.86	0.87	0.85	0.88
Methionine (%)	0.44	0.44	0.49	0.45

Calculation results based on Leeson and Summers (2005), P0: ration contains 8% fish meal; P1: ration with 5% fish meal + 8% fresh BSF; P2: ration with 5% fish meal + 8% dried BSF; P3: ration with 5% fish meal + 8% extract BSF.

Table 2. The average activity and capacity of phagocytosis of peritoneal macrophages against non protein A *Staphylococcus aureus*

Items	Treatment			
	P0	P1	P2	P3
Activity (%)	72.02 ± 1.38^{d}	88.55 ± 0.36^{b}	83.44 ± 0.32^{c}	91.34 ± 0.38^{a}
Capacity (macrophage bacteria -1)	20.77 ± 1.98^{a}	22.25 ± 0.72^{a}	22.44 ± 0.14^{a}	22.84 ± 0.55^{a}

Different superscripts on the same line show significant differences ($P < 0.05$), P0: ration contains 8% fish meal; P1: ration with 5% fish meal + 8% fresh BSF; P2: ration with 5% fish meal + 8% dried BSF; P3: ration with 5% fish meal + 8% extract BSF.

Subsequently, the subcutaneous tissues of the chest and abdomen were ejected, cuts were made to the abdominal muscles along the last rib to open the abdominal cavity. Subsequently, liquid was withdrawn from the peritoneal cavity using a syringe.

Peritoneum liquid was dropped into the glass slide and smeared evenly on its surface, fixed with 100% methanol and air dried for 5 minutes. The slide was stained with 10% Giemsa solution for 25 minutes, then rinsed with distilled water and dried. The preparations were examinated under a microscope using 100 magnification. Phagocytosis activity was calculated best on percentage of macrophage cells that actively phagocyt in 50 macrophage cells. The phagocytic capacity was calculated from the total number of phagocytic bacteria divided by 50 macrophage cells. Two macrophage cells were observed, the exhibiting phagocytic activity and the lacking phagocytic activity against non-protein A *Staphylococcus aureus* bacteria.

Statistical Analysis

Activity and capacity of phagocytic peritoneal macrophages for laying hens were analyzed based on randomized design followed with advanced test using Duncan (Steel & Torrie 1995). Data processing was done using computer software program of Microsoft Excel 2010 and SPSS for Windows version 21.

RESULTS AND DISCUSSION

Effect of using various BSF larvae in the diet had a significant effect ($P<0.05$) on the immunity of laying hens by examining the activity response and phagocytic capacity of peritoneal macrophages exposed to *Staphyllococcus aureus* non protein A.

Macrophages are cells derived from white blood cells present in tissues called monocytes (Abbas et al. 2017). Monocytes and macrophages are phagocytic cells that mainly function in the nonspecific immune system or in innate immunity. Evaluation of the activity and capacity of macrophages in this study was performed *in vitro*. Test results were likely to be higher than in vivo test results because the unique chemotaxis of bacteria in the body produces and accepted by macrophages, making them more active (Wibawan & Soejoedono 2013). Mean phagocytic activity of macrophages in this study was between 72.03 and 91.35%, in line with that reported by Muhsinin et al. (2016) which ranged 76.00 to 90.78% in sentin chicken genotype NRAMP-1 by *Staphylococcus aureus*. Average value of macrophage-phagocytic capacity of 20.77 - 22.84 macrophage-1 bacteria was higher than that reported by Poetri et al. (2008) which was 1.60 macrophage-1 bacteria in commercial single hens (Single Comb Brown Leghorn) at 24 weeks of age and in the same range as that reported by Muhsinin et al. (2016) that was 21.76 - 22.49 macrophages-1 bacteria in Sentul genotypes NRAMP-1. The purpose of the phagocytosis-macrophage capacity test is to detect the ability of a macrophage cell to phagocytose foreign particles or bacterial cells that enter the host body. The P3 treatment has the highest value of phagocyte capacity, respectively is 91.34 ± 0.38% and 22.84 macrophage^{-1} bacteria. This shows that the BSF extract stimulates the macrophage cells to optimally engulf the bacterial cells or foreign particles of the phagocytes.

Results of this study showed that chitin content in various BSF larvae might influence the activity value and phagocytic capacity of macrophages. Comparison of three different species of BSF larvae with different chitin content suggested that: BSF live prepupa phase of 8.72% dry matter (Diener et al. 2009), BSF live pupal phase of 2.7-9.6 g / kg dry matter (Finke 2007: Kroeckel et al. 2012: Bovera et al. 2016), whereas dry BSF and BSF extract did not contain chitin, that lost during processing due to steaming. Chitin is a fibrous protein that is not water soluble but is damaged by denaturation (Klunder et al. 2012). The use of antibiotics might be reduced by the use of BSF larvae, Hong Kong caterpillars and crickets (van Hius et al. 2013).

According to Henry et al. (2015), chitin is a polysaccharide compound found in the arthropod exoskeleton that lacks the chitinase enzyme, making it indigestible to most monogastric animals, including poultry (Sánchez-Muros et al. 2014). Increased population of *Lactobacillus* sp. chitin content of more than 3% in the ration can reduce the population of *Escherichia coli* and *Salmonella* sp. This preserves the health of the chicken digestive tract. Populations of *Lactobacillus* sp. increases when the release of lysosomal enzymes such as β-glucuronidase and β-galactosidase in the digestive tract is also increased, resulting in an increase in the activity value and the phagocytic capacity of macrophages. neutrophils, eosinophils and basophils (Perdigón et al. 2001).

According to Wibawan & Soejoedono (2013), lymphocyte cells produce antibodies in the blood serum that lead to bacterial death. Phagocytosis mediated by heterophilic cells can also lead to bacterial death. Phagocytic cells are divided into three types, namely granulocyte cells (heterophile, eosinophil, and basophil), macrophages and dendritic cells (Devereux 2002). Phagocytes are present in endogenous cells such as neutrophils and eosinophils (polymorphonuclear/PMN), monocytes and in tissues such as macrophages, Kupffer cells, histocytes, microgial cells and alveolar macrophages (Iswara 2012). Phagocytosis is the last stage in a series of processes in a nonspecific immune system in which

immune cells (lymphoid cells) ingest foreign particles or bacterial cells that enter the body (Wibawan & Soejoedono 2013). The formation process of macrophages is when an infection occurs, the immune system activates monocytes that pass from the blood into the tissues. The capacity of monocytes in the form of macrophages is greater than that of heterophile, ie a macrophage cell can phagocytose about 100 bacterial cells (Guyton & Hall 2010).

CONCLUSION

Supplemention using combination of 5% fish meal + 8% extract BSF can stimulate macrophage cells to work optimally in phagocytosis to ingest foreign particles of phagocytes or gram negative bacteria in laying hens.

ACKNOWLEDGEMENT

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Key Words Index

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UDC: 636.084.41

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Pengaruh kandungan protein dan energi metabolis ransum terhadap kinerja produksi, rasio daging terhadap tulang dan komposisi kimia daging ayam SenSi-1 Agrinak (Influence of Dietaryprotein and Energy Levels on Performance, Meat: Bone Ratio, and Meat Chemical Composition of SenSi-1 Agrinak Chickens)

(Org: Eng)

JITV 24(1): 1-8

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 (P0.05) 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.

(Author)

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

UDC: 575.21

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Karakteristik fenotip ayam broiler eksotik, kampung, layer eksotik jantan, KUB-1 dan Pelung (Phenotypic characteristics of Exotic-broiler, Kampung, Male Exotic-layer, KUB-1 and Pelung chickens)

(Org: Eng)

JITV 24(1): 9-14

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.

(Author)

Key Words: Day Old Chick, Morphometry, Organs

UDC: 577.21

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Pengaruh penambahan asam amino terseleksi pada pengencer semen terhadap kualitas dan stabilitas DNA sperma beku sapi Sumba Ongole (The effect of addition selected amino acids in extender semen on quality and DNA stability of frozen-thawed Sumba Ongole bull spermatozoa)

(Org: Eng)

JITV 24(1): 15-21

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 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.

(Author)

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

UDC: 575.21

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Hubungan warna polong dengan kualitas benih *Indigofera zollingeriana* (The relationship of pod colour with the quality of *Indigofera zollingeriana*)

(Org: Eng)

JITV 24(1): 22-28

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: P1 = green, P2 = brownish green, P3 = brown, and P4 = 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 P2 , brownish green pod (14%). The highest number of seeds was in P1 , green pod (5173) and P2 , brownish green pod (4944). The highest germination (62%) was detected in P2 (brownish green). The heaviest sprout was in P2, in brownish green pod (0.035g), highest sprout (2.68 cm) in P4, black pod colour. Based on fungus observation, the black pod (P4) provided the fewest result (6.63%), however most fungus grew very well in P1, 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.

(Author)

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

UDC: 619.616

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Pembuatan antibodi monoklonal-scFV untuk Uji Diagnostik Avian Influenza (Generation of scFv-monoclonal antibody Avian Influenza Diagnostic Tests)

(Org: Eng)

JITV 24(1) 29-38

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.

(Author)

Key Words: Avian Influenza, Nucleoprotein, scFv Antibody, Alternating Panning, POC T

UDC: **619.616**

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Evaluasi strategi penanganan surra untuk kuda dan kerbau di Kabupaten Sumba Timur, Provinsi Nusa Tenggara Timur Indonesia (2010 – 2016) [Evaluation of surra treatment

strategies for horses and buffaloes in East Sumba District, Nusa Tenggara Timur Province of Indonesia (2010 – 2016)].

(Org: Eng)

JITV 24(1): 39-48

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.

(Author)

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

UDC: 575.113

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Identifikasi polimorfisme gen GH|MspI dan GHR|AluI dan hubungannya dengan bobot lahir pedet pada sapi Peranakan Ongole Grati di Loka Penelitian Sapi Potong (Identification of GH|MspI and GHR|AluI gene polymorphism and its Association with calf birth weight of Grati-PO Cattle)

(Org: Eng)

JITV 24(2): 49-55

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

(Author)

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

UDC: 575.113

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Polimorfisme gen SCD1 pada sapi Friesian Holstein di Indonesia (Genetic polymorphism of SCD1 gene of Holstein-Friesian cows in Indonesia)

(Org: Eng)

JITV 24(2): 56-61

Stearoyl-Coenzyme A desaturase 1 (SCD1) belongs to the fatty acid family of desaturases. In lactating ruminants, the SCD1 protein is highly expressed in the mammary gland and is relevant for the fatty acid composition of milk and dairy products. Polymorphism of SCD1 gene in Holstein-Friesian (HF) cows could be used as a basis of molecular selection of cattle in order to increase their productivity. The aim of this study was to investigate the polymorphism of SCD1 gene of Holstein-Friesian cows in Indonesia. A total of 162 blood samples of HF cows were collected from four different locations i.e. Bogor, Sukabumi, Tasikmalaya and Enrekang districts. Genotyping of SCD1 gene used PCR-RFLP method with NcoI restriction enzyme. The result showed that three genotypes (AA, AV and VV) and two alleles (A and V) have successfully found and polymorphic. A allele was dominant in all populations (0.63) and in Hardy

Weinberg Equilibrium. The highest A allele was found in Sukabumi (0.78) and the lowest was in Bogor (0.55). Heterozigosity observed and expected reached 0.471 and 0.470, respectively. In conclusion, genetic polymorphism was found in all population with dominant of A allele. This finding can be used as a early genetic information of Holstein-Friesian cattle in Indonesia and to build breeding strategy for improving of productivity especially improving of healthy fat milk.

(Author)

Key Words: HF Dairy Cow, SCD1, Polymorphisms

UDC: 636.084.52

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Produktifitas sapi Bali yang diberi pakan suplemen dengan molasses yang terdiri dari beberapa tipe agen defaunasi (Productivity of Bali Cattle fed ration supplemented by molasses containing several types of defaunation Agents)

(Org: Eng)

JITV 24(2): 62-67

Livestock animals with relatively fast growth and great body weight are potential as a producer of meat. In Indonesia, the local Muscovy, especially the feathered white is one of the meat-producing livestock. However, an analysis of the growth on local white muscovy is still rarely done. Therefore, the purpose of this study was to determine the point of inflection as growth variables, thus simplifying the preparation of development programs of local white muscovy. A total of 168 of the local unsex white muscovy were examined for their growth since the DOD until 112 days of age. The data of growth i.e. body weight individually examined in every two weeks. The data were analyzed using Gompertz model. The result obtained was the growth equation of local white Muscovy based on the model of Gompertz: Y=2591.3*exp (-3.8636*exp-0.0272*t). Based on these equations, the point of inflection of the local white muscovy occurred at the age of 50 days with a weight of 953.29 g. The maximum body weight gain achieved was 2591.30 g. It is concluded that the growth of local white muscovy was relatively slow, but the body weight was very heavy.

(Author)

Key Words: Local White Muscovy, Growth, Starter Period, Grower Period

UDC: 636.085.51

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Upaya meningkatkan fermentasi rumen *Panicum maximum* dengan suplemen biologis secara in vitro (Enhance in-vitro rumen fermentation of *Panicum maximum* with biological supplements)

(Org: Eng)

JITV 24(2): 68-72

Recently the utilization of biological feed additives over chemical feed additives in animal feeds have increased. The objective of the present study was to evaluate the effect of supplementing wild guinea grass (*Panicum maximum*) with two plant species, *Artocarpus heterophyllus* (jack leaves; ah) and *Tridax procumbens* (TP) containing plant secondary metabolites tannin and saponin, respectively and the enzyme product dyadic cellulase (CE) and yeast (YE). For each suplement two levels of treatments were tested. In plant-based suplements 20 (AHT1, Tpt1) and 30% (AHT2 and Tpt2) substituted the base substrate. The enzyme was applied as 10 µl (CET1) and 20 µl (CET2) and yeast as 4 mg (YET1) and 6 mg (YET2). the experimental design was a randomized complete block design (RCBD) and the period of in vitro rumen fermentation incubation was 72 hrs. All treatments significantly (P<0.05) enhanced the *in vitro* gas production (IVGP) compared with the control. Treatments of ah and ce significantly (P<0.05) improved the *in vitro* rumen dry matter degradability (IVRDMD). All treatments significantly (P0.05) influenced with supplements. in conclusion, treatments enhanced the rumen fermentation in means of enhanced IVGP, IVRDMD and reduced protozoa numbers.

(Author)

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

UDC: 636.32

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Ciri-ciri domba dan efek suplemen protein pada profil semen di domba asli Bangladesh (Traits of Sheep and Effects of Protein Supplements on Semen Profile in Indigenous Sheep of Bangladesh)

(Org: Eng)

JITV 24(2): 73-80

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

(Author)

Key Words: Sheep, Traits, Protein supplements, Semen quality

UDC: 637.057

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Kualitas daging ayam jantan Sentul dengan konsentrasi imunoglobulin kuning telur yang berbeda (Meat Quality on Sentul Cocks with Different Immunoglobulin Yolk Concentrations.)

(Org: Eng)

JITV 24(2): 81-86

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

(Author)

Key Words: Sentul Cocks, IgY, Meat Quality

UDC: 577.175.3/.7

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Profil plasma β-endorphin dan kortisol sekitar periode *peripartnutrient* pada kondisi stres pada kerbau Mesir (Plasma β-endorphin and cortisol profiles around periparturient period at stressful conditions in Egyptian buffalo)

(org: Eng)

JITV 24(3): 101-113

The study purpose was to determine the relationship between reproductive disorders as a stress factor with plasma β–endorphin and cortisol in buffalo around parturition and changes in these parameters could be used as an objective measure of the stress associated labour. The periparturient period, the period immediately before and after calving, is a challenging time for dairy cattle that must cope with physiological, metabolic and endocrine changes. β-endorphin and cortisol rapidly increased in response to stress in cattle. The study determined the level of blood plasma β-endorphin and cortisol of buffalo with reproductive disorders (dystocia and retained placenta) and Low body condition score during periparturient period. Twenty multiparous Egyptian buffalo at late pregnancy period were used for two months before parturition. β-endorphin concentrations were higher in reproductive disorders groups. Whereas, β-endorphin concentrations were 134.9±4.8 for RP, 121.3±4.9 for dystocia, 114.2±8.4 for Low BCS and 113.5±6.5 pg/ml for control. At the closer period around parturition both of plasma β-endorphin and cortisol followed the same trend toward a gradually increased value during -2,-1days and zero time in all groups. A concomitant trend was noticed in β-endorphin and cortisol concentrations in postpartum period with values decreased were observed in all groups after parturition continued for month or more. Buffalo with reproductive disorders were showed a high relative values in β-endorphin and cortisol. Significant differences (P≤0.01) were observed between the groups. Generally, buffaloes with

reproductive disorders had a clear impact on blood plasma β-endorphin around parturition process.

(Author)

Key Words: Buffalo, β-endorphin, Cortisol, Dystocia, Retained Placenta

UDC: 577.175.3/.7

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Pengaruh penambahan Insulin-Transferrin-Selenium pada pematangan dan fertilisasi *in vitro* oosit sapi Bali (Effect of the addition of Insulin-Transferrin-Selenium on *ın vitro* maturation and fertilization of bali cattle oocytes)

(Org: Eng)

JITV 24(3): 95-102

This study was conducted to determine effect of the addition of Insulin Transferrin Selenium (ITS) on in vitro maturation and fertilization of Bali cattle oocytes. Bali cattle ovary is sliced to obtain oocytes, then oocytes were collected and selected based on their quality. Oocyte then maturated for 24 hours and fertilized for 18 hours in an incubator of 5% CO2 and 38.5°C. Oocyte was stained with 2% acetoorcein, then observed under a microscope. This study was done based on a Completely Randomized Design (CRD) with four treatments of ITS addition (P0 control; P1 (5 ng/ml); P2 (10 ng/ml); and P3 (15 ng/ml)) in 4 replications. Parameters observed were the stage of oocyte maturation level consisting of germinal vesicle (GV), germinal vesicle break down (GVBD), metaphase-I (MI) and metaphase-II (M-II), fertilization rate (0 pronucleus (0 PN) , 1 pronucleus (1 PN), 2 pronucleus (2 PN) and more than 2 pronucleus (>2 PN)). Results showed that the highest percentage of oocyte maturation rate tends to be in the M-II stage, which is achieved by P1 oocytes with ITS addition as much as 5 ng/ml while the highest percentage of fertilization rate at PN-2 stage, which was produced by P3 oocytes with ITS addition as much as 15 ng/ml. It is concluded that the addition of ITS at 5 ng/ml tends to produce the best maturation rate and for the best level of fertilization tends to be as much as 15 ng/ml of ITS addition.

(Author)

Key Words: Bali Cattle Ovary, Fertlization, Insulin-Transferrin-Selenium, Maturation

UDC: 57.017.5

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Retensi plasenta dan hubungannya dengan komponen darah pada sapi Mesir persilangan (Retained placenta in Relation with Blood Components in Egyptian crossbred cattle)

(Org: Eng)

JITV 24(3): 103-111

Retained placenta (RP) is one of the main reproductive disorders in dairy cattle and happened if the placenta is not released out within certain duration around 12 h post calving and 3 h post foaling. The present study was carried out in the veterinary units on 14 normal cows and 32 cows with RP. Cows were chosen after about 6 to 12 h from parturition in Menoufia governorate, Egypt. Results show that there was significant increase of concentrations of blood components in normal cows than those in RP cows except in globulin, sodium and manganese. Plasma concentrations of biochemical components were significant between summer and winter in cholesterol, total protein and albumin but it was no difference in glucose, globulin and A/G ratio. Plasma concentration is higher in winter than in summer in all macro elements except potassium and Ca/P ratio. Also, Plasma concentration is higher in winter than in summer in all micro elements except Manganese. Plasma concentrations of biochemical components were not significant between parities except in A/G ratio. No significant in plasma concentration between summer and winter in all macro elements. But, there was significant deference ($P \leq 0.01$) in plasma concentration of Cu and Cd between summer and winter, and it was significant deference ($P \leq 0.05$) in Fe and Se but, there was no significant in Co, Zn, Mn, and Mo.

(Author)

Key Words: Cattle, Macro-Micro-Minerals, Plasma Constituents, Retained Placenta

UDC: 577.112.6

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Aktivitas antimikroba dan anti-inflamasi fraksi dan peptida tunggal derivat dari protein susu kuda (Antimicrobial and anti-inflammation activities of peptide fraction and purified peptides derived from mare milk)

(Org: Eng)

JITV 24(3): 112-121

Mare milk protein contains bioactive peptide which is beneficial for human and animal health. Peptides in the fraction and single may show different activities. The objectives of the study were to evaluate antimicrobial and anti-inflammation activities of the fraction and single peptide derived from mare milk protein. Antimicrobial assay was conducted by testing antibacterial and antifungal activities of fraction <3 kDa, single peptide LVNELTEFAK (peptide 1), HPYFYAPELLYYANK (peptide 2), and LANSLTEFAK (peptide 3) against *Escherichia coli* and *Candida albicans*.

Antioxidant assay was conducted using 2,2'-*azino-bis* (3-*ethylbenzthiazoline*-6-*sulphonic acid* (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. Anti-inflammation effect was detected by interleukin 1-β (IL- 1β) and Tumor Necrosis Factor -α (TNF-α) production in mice after administration of *Escherichia coli's* lipopolysaccharide (LPS) and combined with fraction or single peptide. Result showed that peptide in fraction form has higher antibacterial, antifungal activities and antioxidant activities against radical ABTS than all of single peptide ($P<0.05$). Anti-inflammation activity was showed by peptide 1 and peptide 2 which was indicated by significantly decreasing of IL-1β after treatment ($P<0.05$). Based on the results, it was concluded that antimicrobial and anti-antioxidant activities fraction <3 kDa was better than single peptide. For anti-inflammation activity, a single peptide showed better activity than fraction.

(Author)

Key Words: Antimicrobial, antioxidant, anti-inflammation, peptide, mare milk

UDC: 577.112.7

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Antigenik karakterisasi pada protein M2e menggunakan antibodi monoklonal anti-M2 dan antibodi poliklonal anti-M2e (Characterisation of M2e antigenicity using anti-M2 monoclonal antibody and anti-M2e polyclonal antibodies)

(Org: Eng)

JITV 24(3): 122-134

Ectodomain matrix 2 protein (M2e) is a potential antigen for detection of influenza-A-virus infection among vaccinated birds (DIVA test). However, the antigenicity and immune response induced by M2e in either humans or animals are poorly understood. Seventeen M2e peptides and sixteen recombinant M2e (rM2e) proteins with amino acid (aa) changes introduced at position 10, 11, 12, 13 14, 16, 18 and 20 were compared by western blot (WB) and enzyme-linked immunosorbent assay (ELISA) using mouse anti-M2 monoclonal antibody (mAb) 14C2, and chicken- or rabbit-polyclonal antibodies (pAb). The mAb 14C2 had the best discriminating power and aa position 11 was the important immunodominant for mAb14C2, that affected binding to a greatest degree. Changes in the adjacent position 14, 16 and 18 also influenced the binding, and it detected regardless of the method (WB or ELISA), or the antigen used (M2e peptide or rM2e). For chicken pAb and rabbit pAb, the immunodominant aa was position 10 and the antibody reaction was not affected by aa change at 11. The binding of rabbit pAb was also affected by changes at 14 and 16, which confirm the contribution of these positions to the M2e antigenicity. Position 10 was the only important position for the binding of chicken pAb to M2e. Overall, the study showed that the M2e antigenic sites are located between residues 10 – 18 and that aa changes at position 10, 11, 12, 14, 16 and 18 may all affect the antibody binding within the M2e protein.

(Author)

Key Words: Antigenicity, Influenza A Virus, M2e Epitope

UDC: 631.151

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Identifikasi sumber daya dalam sistem usaha peternakan broiler (Identification of resources in the system of broiler farming business)

(Org: Eng)

JITV 24(3): 135-142

Accessibility of resources in theory can affected the development of broiler farming in a region. This research was conducted with the objectives to formulate indicators of resource wich is influence to the development of broiler farming business. The research was conducted in August 2017 up to January 2018 in Malang District of East Java Province, Indonesia. The number of sample is 100 respondents of broiler farmer was participated in this research. The observed variables consist of: (a) financial resources, (b) technology resources, (c) physic resources, (d) economy resources, (e) environmental resources, (f) social resources, (g) human resources, and (h) business development. The data was analyze used by SEM with SmartPLS 2.0 analysis tool. The results indicate that the development of broiler farming business is directly influenced with significant value by the financial, physic, economic, and the human resources, but not affected by technology, environmental, and social resources. The development of broiler farming business is indirectly influenced through quality of human resources is affected by the financial, technology, physic, and economy resources. The conclusion of this research is that technology resources play an important role indirectly, because it must be supported by human resources in the model development of broiler farming business.

(Author)

Key Words: Livestock Business Resources, Business Development, Human Resources, Broiler Chicken

UDC: 575.113.1

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Pengukuran hubungan genetik antara sifat-sifat pertumbuhan dan produksi susu Kerbau Mesir (Assessment of genetic relationships between growth traits and milk yield in Egyptian buffaloes)

(Org: Eng)

JITV 24(4): 143-150

Data in this study were collected from live body weight records and milk yield for the first three lactations of Egyptian buffaloes maintained at the Mahallet Mousa Experimental Station of Animal Production Research Institute, relying on 987 records of Egyptian buffaloes spread over 16 years. These data were analyzed to estimate genetic parameters using animal model. Overall means in kilograms of BW, WW, W18, WFC, 1stMY, 2ndMY and 3rdMY were 36.56, 96.95, 322.02, 462.09, 1561.53, 1755 and 1837.71, respectively. Direct additive heritability (h^2a) for mentioned traits were 0.31, 0.22, 0.24, 0.27, 0.23, 0.23 and 0.17, respectively. Corresponding computation of maternal heritability (h^2m) for same traits were 0.39, 0.34, 0.22, 0.40, 0.29, 0.31 and 0.21, respectively. Evaluation of genetic correlations among different all studied traits were positive and ranged from 0.07 to 0.83, while phenotypic correlations were positive and ranged from 0.02 to 0.55. Accuracy of (PBV's) varying from 62 to 76, 62 to 83 and 41 to 77% for sires, cows and dams, successively; pointing out the genetic improvement could be achieved through any pathway of them. Higher direct and maternal heritability for BW and WFC and genetic correlations between first three lactations milk yield and each of BW and WFC higher than genetic correlations between first three lactations milk yield and WW and W18. Therefore, it is appropriate to select buffalo female calves for live body weight at birth than for live body weights at other ages.

(Author)

Key Words: Breeding Values, Egyptian Buffaloes, Genetic Parameters, Growth Traits, Milk Yield

UDC: 636.58.034

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Produktivitas ayam petelur KUB-2 generasi ke-4 (The productivity of 4th generation KUB-2 chicken)

(Org: Eng)

JITV 24(4): 151-157

KUB-2 line of chicken has improved local chicken selected from the KUB-1 chicken line. KUB-2 was selected for more egg production and yellow shank. KUB-1 chicken has 64% various of black feather color, which sometimes tends to have unpreferred dark carcass. Yellow shank color has a positive correlation with the skin color of carcass. As many as 517 pullets of KUB-2 at 4th generation were divided into two groups of 194 pullets of KUB-2_{kk} (yellow shank) and 323 pullets of KUB-2_{nk} non-yellow shank). The chickens were raised intensively in the individual cages for the 24 weeks observation. Variables measured were age at first egg (AFE) bodyweight at first egg (BWFE), egg weight at first egg (EWFE), average egg weight (AEW), average egg production (AEP) during 24 weeks, feed conversion ratio (FCR) of 25-43 weeks of age, and mortality. The result showed that there was no statistically significant different ($p>0.05$) between KUB-2_{nk} and KUB-2_{kk} respectively for AFE of 156.2 d and 158.1 d, for BWFE of 1788 g and 1808 g, for EWFE of 31.32 g and 31.34 g, for AEP_{24} of 103.3 eggs or 61.5% and 101.9 eggs or 60.7%, and for $FCR_{25\text{-}43}$ of 3.53 and 3.54. AEW increased with increasing age of hen, the mortality of the whole population was 0.98%.

(Author)

Key Words: 4th Generation KUB-2 Chicken, Egg Production, Yellow Shank

UDC: 577.15

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Optimasi produksi mananase BS4 pada ketebalan substrat dan jenis baki yang berbeda (Optimization of BS4 enzyme production with different substrate thickness and type of trays)

(Org: Eng)

JITV 24 (4): 158-165

BS4 enzyme that is produced from solid substrate fermentation (SSF) on coconut cake with *Eupenicillium javanicum* BS4 in tray bioreactor has been applied as a feed additive. It increases the nutritional value of animal feedstuff. The BS4 production on SSF may be influenced by the better aeration through the perforated trays or by the thinner substrate. The aim of this research is to optimize the production of BS4 with different substrate thicknesses and types of trays. The trial was carried out using a factorial randomized design (2x2x3) with 6 replicates. The first factor was the type of trays: i.e., non-perforated and perforated tray. The second factor was the thickness of the substrate: i.e., 1.5 and 3.0 cm, while the third factor was the duration of fermentation: i.e. 5, and 7 days. The variables observed were moisture content, dry matter loss (DML), mannanase and saccharification activities, soluble protein content, their specific activities, and yield. Statistical analysis showed no interactions between the three factors, but there were interactions between types of trays and substrate thicknesses, as well as type of trays and incubation times on the mannanase activity and yield of mannanase. The results showed that DML was observed on day 7 were around 31.43- 36.89. The highest mannanase activity was observed on the non-perforated tray with 3 cm thickness on day 7. The saccharification activity towards palm kernel meal was better in the non-perforated tray on day 7 but not influenced by The yield value of mannanase and saccharification activities on a non-perforated tray with 3.0 cm thickness on day 7 was also the highest. Based on energy efficiency and the cost of production, it can be concluded that the optimum condition to produce the BS4 enzyme was observed in the non-perforated tray with 3 cm thickness and fermented for 7 days.

(Author)

Key Words: *Eupenicillium javanicum* BS4, Mannanase, Perforated tray, Solid Substrate Fermentation, Thickness

UDC: 636.084.52

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Penggunaan kulit kopi sebagai pengganti rumput gajah pada performa sapi Madura (The use of coffee husk as napier grass substitution and its effect on Madura cattle performance)

(Org: Eng)

JITV 24(4): 166-172

This research was aimed to evaluate the performance of fattened Madura cattle fed on coffee husk as a source of fibre to substitute grasses. Twenty Madura steers aged approximately 1.5 to 2.0 years with initial weight of 165-190 kg were divided randomly into four different experimental diets, namely R0= 90% concentrates + 0% coffee husk + 10% napier grass, R1= 90 % concentrates + 3.33% coffee husk + 6.67% napier grass, R2 = 90% concentrate + 10% 6.67% coffee husk + 3.33% napier grass, R3 = 90% concentrates 90% + 10% coffee husk + 0% napier grass. The feed was given at 3% body weight of dry mater. The cows were kept in individual pen for two months. The variables measured were dry mater intake, feed efficiency, average daily gain (ADG), the digestibility of feed and income over feed cost. The data obtained were analyzed using analysis of variance from the randomized block design followed by Duncan's multiple range test. The results showed that dry matter intake, ADG, feed efficiency and feed digestibility of cattle on each treatment of the feed were not significantly different (P > 0.05). The average of dry matter intakes and daily gain for R0, R1, R2, and R3 were 6.01, 5.84, 5.73 and 5.62 kg/head/day and 0.88, 0.87, 0.84 and 0.93 kg/head/day respectively. While the average of feed efficiency and DM digestibility for R0, R1, R2, and R3 were 14.64, 14.89, 14.65 and 16.54 % and 84.82, 84.37, 83.47 and 83.30 %, respectively. It is concluded that the used of 10% coffee husk as a source of fibre for substitution of napier grass can be used without negative effect on madura's cattle performances and tend to give higher values of IOFC on fattening program.

(Author)

Key Words: Coffee Husk, Feed Utilization, IOFC, Madura Cattle Performance, Napier Grass

UDC: 636.085.51

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Aktivitas *Rhizopus oligosporus* dalam bentuk ekstrak kasar dan serbuk untuk menurunkan *Aspergillus flavus* dan aflatoksin pada jagung (*Rhizopus oligosporus* activity in crude extract and powder form to reduce *Aspergillus flavus* and aflatoxin in corn)

(Org: Eng)

JITV 24(4): 173-181

Rhizopus oligosporus (RO) in isolate culture was known to reduce contamination toxigenic mold *Aspergillus flavus* (AF) and aflatoxin B1 in chicken feed. Application in culture form was not effective. The aim of this research was to evaluate RO activities in extract and inoculum form to reduce contamination of AF and aflatoxin B1 in corn. RO was harvested from agar plate, blended, added with water (ratio 1:1 (w/v)) and centrifuged. Supernatant was filtered using Whatman 41. Inoculum was made by inoculation RO in soy powder and incubated at 28oC for 5 days. Inoculum was dried at 40oC for 24-28 hours. Assay was conducted by addition extract or inoculum to corn. Extract and corn ratio were 1:1 (v/w), while inoculum doses were 5, 25, 50, 1000 dan 200 g/kg corn. Assay for aflatoxin B1 was done using kit ELISA aflatoxin. The result of this research showed that extract was able to reduce AF contamination up to 1 log 10, while the less concentration of inoculum which able to inhibit AF up to 6 log 10 was 100 g/kg corn. Extract RO 125 and 250 mL/kg corn was able to reduce aflatoxin contamination by 93.69 % and 85.84 %. Inoculum at dose 5 and 100 g/kg corn was able to reduce aflatoxin 57.58% and 85%. Based on the result, it could be concluded that RO in extract or inoculum form was able to reduce contamination of AF and aflatoxin B1 in corn. *Rhizopus* as inoculum was easier to be applied than in extract form.

(Author)

Key Words: Aflatoxin, *Aspergillus flavus*, Extract, Powder, *Rhizopus oligosporus*

UDC: 636.083.5

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Suplementasi Black Soldier Fly (*Hermetia illucens*) untuk aktivitas dan kapasitas makrofag fagositosis pada ayam petelur [Supplementation of Black Soldier Fly (*Hermetia illucens*) on activity and capacity phagocytic macrophage of laying hens]

(Org: Eng)

JITV 24(4): 182-187

Black Soldier Fly are natural antibiotics. It is expected that the use of BSF larvae in poultry rations as an alternative source of conventional protein will contribute to improving the immune status and maintaining animal health, thereby reducing the use of antibiotic growth promoters (AGPs). This study aims to evaluate the effect of the best type of BSF

protein for determining the health status of laying hens based on the activity and capacity of macrophage phagocytosis on the non-protein A bacterium *Staphyllococcus aureus*. The results of this study indicate that the BSF extract (P3) has the highest phagocytic capacity value. This proves that the BSF extract can induce macrophage cells to optimally process bacterial cells or foreign phagocyte particles. The average activity value and phagocytic capacity of peritoneal macrophages showed the highest BSF extract (P3), respectively is 91.34 ± 0.38% and 22.84 macrophage^{-1} bacteria.

(Author)

Key Words: Activity and Capacity Of Phagocytic Peritoneal Macrophages, Antibiotic Growth Promoters (AGPs), Black Soldier Fly (BSF)

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   a. Lawrence TLJ, Fowler VR. 2002. Growth of farm animals. 2nd ed. New York (USA): CABI Publishing.
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   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.

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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.

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