ISSN 0853-7380
E-ISSN 2252-696x

Accredited by LIPI
Certificate Number: 655/AU3/P2MI-LIPI/07/2015

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 21
Number 2
June 2016

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

JITV	Volume 21	Number 2	Page 73-150	Bogor, June 2016	ISSN 0853-7380

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

JITV	Volume 21	Number 2	Page 73-148	Bogor, June 2016	ISSN 0853-7380 E-ISSN 2252-696X

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

Collaborated with:
Indonesian Society of Animal Science

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

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

PREFACE

In this edition, volume 21 no. 2 June 2016, we proudly present articles from various disciplines such as animal reproduction, feed, nutrition technology, forages technology, and veterinary technology. The articles published in this edition are: "Estimated variance components and breeding values for pre-weaning growth criteria in Romney sheep"; "*In vitro* rumen fermentation characteristics and microbes of thin tail sheep given sweet potato biomass"; "Effect of different protein and energy levels in concentrate diets on nutrient intake and milk yield of Saanen x Etawah Grade goats"; "Histological changes of liver tissue and serobiochemical relation in does with pregnancy ketosis"; "Methane emission factors for enteric fermentation in beef cattle using IPCC Tier-2 method in Indonesia"; "Greenhouse gas emissions from cattle production sector in South Korea"; "Effect of addition of vitamin C in diluent medium for quality of dairy cow spermataozoa after thawing"; "Productivity of *Indigofera zollingeriana* under different canopy and soil acidity level in oil palm estate" and "Determination of fibernolytic enzymes activity of white rot fungi isolated from oil palm fronds".

Hopefully these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from other authors to contribute their papers to Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor;

Bogor, June 2016

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

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 21, Number 2, June 2016 ISSN 0853-7380 E-ISSN 2252-696X

LIST OF CONTENT

Estimated Variance Components and Breeding Values for Pre-Weaning Growth Criteria in Romney Sheep

Faid-Allah E[1], Ghoneim E[1], Ibrahim AHM[2]

[1]*Department of Animal Production, Faculty of Agriculture, Minoufiya University, Egypt*
[2]*Department of Animal Breeding, Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt*
E-mail: ifaidallah@yahoo.com

(received 24-04-2016; revised 17-05-2016; accepted 09-06-2016)

ABSTRAK

Faid-Allah E, Ghoneim E, Ibrahim AHM. 2016. Estimasi komponen varian dan nilai pemuliaan kriteria pertumbuhan pra-sapih pada domba Romney. JITV 21(2): 73-82. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1353

Penelitian ini dilakukan untuk melihat komponen-komponen ragam, pengaruh genetik langsung, parameter genetik maternal, estimasi nilai pemuliaan (EBV) dan faktor-faktor yang mempengaruhi kriteria pertumbuhan pra-sapih pada domba Romney. Data yang diperoleh merupakan data periode tahun 2006-2012 dari 4989 ekor anak domba yang merupakan keturunan dari 76 domba Romney jantan dan 2190 domba Romney betina yang dibudidayakan di S. Island, New Zeland melalui Gene Marker Laboratorium, Fakultas Pertanian dan Ilmu Hayati (Faculty of Agriculture and Life Science), Universitas Lincoln, New Zeland. Hasil penelitian membuktikan bahwa faktor genetik dan non-genetik memiliki pengaruh nyata ($P<0.05$) terhadap faktor-faktor kriteria yang diteliti. Estimasi genetik dan lingkungan bobot hidup saat lahir (LBW), saat penyapihan (LWW) dan rasio Kleiber (KR) berturut-turut adalah sebesar 0.20±0.074, 0.15±0.042 dan 0.14±0.052 untuk heretabilitas langsung (h^2a±SE); 0.59±0.219, 0.41±0.023 dan 0.08±0.002 untuk heritabilitas maternal (h^2m±SE); 0.11684, 2.6378 dan 0.27565 untuk ragam aditif (σ^2a); 0.34596, 7.1179 dan 0.14532 untuk ragam maternal (σ^2m); dan 0.002395, 10.1262 dan 0.509339 untuk ragam lingkungan tetap (σ^2e). EBV dari LBW, LWW dan KR berturut-turut berkisar antara -0.555 : 0.502, -1.554 : 3.006 dan -0.633 : 0.242 untuk pengaruh genetik langsung, -0.863 : 0.954, -4.942 : 2.554 and -0.469 : 0.179 untuk maternal domba jantan serta -0.664 : 0.830, -2.996 : 4.586 dan -1.651 : 0.677 untuk pengaruh genetik langsung, 1.429 : 1.142, -7.541 : 4.920 dan -1.223 : 0.492 untuk maternal domba betina. Hasil penelitian ini juga menunjukkan pentingnya mempertimbangkan faktor-faktor non-genetik dalam performan pertumbuhan pra-sapih anak domba. Heritabilitas sedang dan koefisien positif dari fenotipe dan hubungan genetik antar kriteria yang diteliti berpeluang untuk dapat dikembangkan melalui seleksi tradisional.

Kata Kunci: Domba Romney, Faktor Genetik, Faktor Non-Genetik, Heritabilitas, Pertumbuhan Pra-Sapih, EBV

ABSTRACT

Faid-Allah E, Ghoneim E, Ibrahim AHM. 2016. Estimated variance components and breeding values for pre-weaning growth criteria in Romney sheep. JITV 21(2): 73-82. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1353

This study was carried out to investigate variance components, direct heritability, maternal genetic parameters, estimated breeding values (EBV) and factors affecting pre-weaning growth criteria of Romney sheep. Data were collected over the period from 2006 to 2012 with records of 4989 lambs descended from 76 rams and 2190 ewes of Romney sheep maintained at S. Island of New Zealand via Gene Marker Lab., Faculty of Agric. and Life Sci., Lincoln Univ., New Zealand. Results proved that genetic and non-genetic factors affecting studied criteria had significant effects ($P<0.05$). Genetic and environmental estimates of live body weights at birth (LBW), weaning (LWW) and Kleiber ratio (KR) were 0.20±0.074, 0.15±0.042 and 0.14±0.052 for direct heritability (h^2a±SE); 0.59±0.219, 0.41±0.023 and 0.08±0.002 for maternal heritability (h^2m±SE); 0.11684, 2.6378 and 0.27565 for additive variances (σ^2a), 0.34596, 7.1179 and 0.14532 for maternal variances (σ^2m); and 0.002395, 10.1262 and 0.509339 for permanent environmental variances (σ^2e), respectively. EBV's of LBW, LWW and KR ranged from -0.555: 0.502, -1.554: 3.006 and -0.633: 0.242 direct, -0.863: 0.954, -4.942: 2.554 and -0.469: 0.179 maternal for rams, respectively; and -0.664: 0.830, -2.996: 4.586 and -1.651: 0.677 direct, 1.429: 1.142, -7.541: 4.920 and -1.223: 0.492 maternal for ewes, respectively. Results suggest the importance of considering the non-genetic factors in pre-weaning growth performance of lambs. Moderate heritability and positive coefficients of phenotypic and genetic correlation for studied criteria indicate to the possibility of improving them using traditional selection.

Key Words: Romney Sheep, Genetic Factors, Non-Genetic Factors, Heritability, Pre-Weaning Growth, EBV

INTRODUCTION

Growth potential of lambs is very important in the sheep production. It is essential to have knowledge of genetic parameters for these economically important criteria to formulate breeding strategies for better production (Gowane et al. 2015). The relative importance of direct and maternal additive genetic effects for growth should be considered when sheep producers formulate their breeding plans (Talebi et al.

2007). Maternal genetic effects arise from allelic differences between individual mothers at loci influencing offspring phenotype and are a heritable component of phenotypic variance themselves. It can dampen response to the selection, when direct-maternal genetic covariance is negative, or accelerate, when direct-maternal genetic covariance is positive (Wolf et al. 1998). Maternal care and genetic potential of lambs are two factors affecting the early growth of lambs. In the pre-weaning period, animals are fed by their mother's milk (Ghafouri-Kesbi 2013). Growth criteria are influenced by many factors includes additive genetic effects, maternal genetic effects and non-genetic factors (Farokhad et al. 2010). Adjustments of data for environmental factors, such as year or/and season of birth, parity of ewe, type of birth, sex of lamb and location are necessary to obtain reliable estimates and to increase the accuracy of selection of breeding animals (Thiruvenkadan et al. 2011). Studies of estimated breeding value, variance components, genetic parameters, and factors affecting growth criteria in New Zealand Romney sheep were infrequent for the last decade. However, Sheep production has been the important source of sustainable livelihood of the rural folk in New Zealand.

A strategy to increase the efficiency of meat production in both the traditional and intensive systems is the selection of animals regarding the efficiency of feed utilization. Since individual sheep differ in their ability to utilize feed efficiently, selecting the most efficient animals, those with lower maintenance requirements, results in a significantly lower production cost (Ghafouri-Kesbi et al. 2011). Direct selection for lower maintenance requirements is difficult. However, measures of feed efficiency such as Kleiber ratio would be used to achieve this goal (Arthur et al. 2001). Estimated Breeding Value (EBV) is a prediction of the genetic merit of an animal for selection criteria such as growth, milk, and prolificacy. EBVs increase the accuracy of selection for superior performance.

This study investigated genetic and non-genetic factors affecting pre-weaning growth criteria of New Zealand Romney sheep breed and estimate its breeding values, variance components, direct, and maternal genetic parameters.

MATERIALS AND METHODS

Animal data

Data were collected over the period from 2006 to 2012 except 2011 with records for studied criteria on total of 4989 pedigree lambs descended from 76 rams and 2190 ewes from 14 commercial farms of Romney sheep maintained at S. Islands of New Zealand via Gene Marker Lab., Faculty of Agric. and Life Sciences, Lincoln Univ., New Zealand. The pedigree data included birth and weaning weight and its dates, sex of lamb, ewe parity, and type of birth. Lambs were weaned at about three months of age. Studied pre-weaning growth criteria were live body weights at birth (LBW), weaning (LWW) and Kleiber ratio (KR = ADG_LWW/$LWW^{0.75}$). Kleiber ratio is defined as growth rate divided by body mass powered 0.75 [metabolic weight] (Kleiber 1947). The breeding season started from February to late May. Those selected rams and ewes were divided into mating groups to avoid inbreeding. Ewes were joined in a pen with a single ram in a group of 40-50 ewes. After mating, ewes were separated from rams and kept as one group until the lambing season which usually starts in August to October.

Statistical analysis

Data were analyzed by general linear model via Statistical Analysis System [SAS] (SAS 2002), to estimate effect of ram, ewe within ram, year of birth, parity of ewe, sex of lamb, type of birth and farm on three pre-weaning growth criteria: LBW, LWW, and KR. Statistical models used to analyze pre-weaning growth criteria may be written as the following formula:

$$Y1_{ijklmnop} = \mu + S_i + D_j + Y_k + P_l + G_m + B_n + F_o + e_{ijklmnop}$$

$$Y2_{ijklmnop} = \mu + S_i + D_j + Y_k + P_l + G_m + B_n + F_o + \beta_{(age\ at\ weaning)\ ijklmno} + e_{ijklmnop}$$

where:

$Y1$ = the observed records on the criterion (LBW)
$Y2$ = the observed records on the criteria (LWW,KR)
μ = the overall mean
S_i = the random effect of i^{th} ram, i= 1,., 76,
D_j = the random effect of j^{th} ewe within ram, j= 1,., 2190
Y_k = the fixed effect of k^{th} year of birth, k = 1,.,6
P_l = the fixed effect of l^{th} parity of ewe: l= 1,.., 3
G_m = the fixed effect of m^{th} sex of lamb, m = 1, 2
B_n = the fixed effect of n^{th} type of birth, n = 1, …, 3
F_o = the fixed effect of o^{th} farm, o= 1, …, 14
β_{agew} = the linear regression coefficient of body weight on age at weaning as a covariate
$e_{ijklmno}$ = residual random error.

Estimation of variance components, direct and maternal genetic parameters and estimated breeding value (EBV) via BLUP (Best Linear Unbiased Prediction) were carried out by derivative-free REML with a simplex algorithm using the Multiple Trait Derivative-Free Restricted Maximum Likelihood [MTDFREML] (Boldman et al. 1995). Models in matrix notation were as follow:

$$Y_1 = X\beta_1 + Z_a a + Z_m m + Z_c c + e$$

$$Y_2 = X\beta_2 + Z_a a + Z_m m + Z_c c + e$$

where:

Y_1	=	vector of observations (LBW)
Y2	=	vector of observations (LWW, KR)
X	=	incidence matrix for fixed effects and covariates
β_1	=	vector of fixed effects (i. e. year of birth, parity of ewe, sex of lamb, birth of rank, farm)
β_2	=	vector of fixed effects and covariates (i. e. fixed effect = year of birth, parity of ewe, sex of lamb, birth type, and farm; covariate = age at weaning)
Z	=	incidence matrix for random effects
a, m, c	=	vector of random effects (additive genetic, maternal genetic and permanent environmental effect)
e	=	vector of residual effects (0, I σ^2_e).

RESULTS AND DISCUSSION

Descriptive statistics

Table (1) shows the mean, standard deviation (SD) and coefficient of variability (CV) of studied pre-weaning growth criteria. Live body weights at birth, weaning and Kleiber Ratio were 5.573±0.8848 kg (CV= 15.88%), 32.48±5.7534 kg (CV= 17.71%) and 22.39±2.2246 (CV= 9.93%) for 4989 records of pedigreed Romney lambs weaned at 88.13±6.75 days, respectively.

Body weights at birth and weaning in Romney lambs literature were 4.10±0.76, 4.32, 4.85±0.607 and 5.97±0.02 kg of birth weight (Morris et al. 1996; Morris et al. 2000; Everett-Hincks et al. 2014; Ibrahim 2015, respectively); and 28.7±3.1, 28.71 and 36.81±0.11 kg of weaning weight (Morris et al. 1996; Morris et al. 2000; Ibrahim 2015, respectively).

In other sheep breeds, body weights were 2.8, 3.36±0.65, 4.12±0.65, 4.02±0.85 and 3.05±0.54 kg at birth and 17.8, 12.69±2.38, 20.72±4.33, 21.65±5.53 and 14.53±3.20 kg at weaning, in Zimbabwe Sabi lambs (Matika et al. 2001), in Egyptian Farafra lambs (Mousa et al. 2006), in Iranian Zandi lambs (Ghafouri-Kesbi & Eskandarinasab 2008), in Arman lambs (Farokhad et al. 2011) and in Indian Malpura lambs (Gowane et al. 2015), respectively. Ghafouri-Kesbi (2013) reported that statistics of Kleiber Ratio at pre-weaning period were 19.61±2.4 (CV= 12.2%) in fat-tailed Mehraban lambs.

Genetic and non-genetic factors

Table (2) shows that rams and ewes as random factors significantly (P≤0.01) affected the studied pre-weaning growth criteria in Romney sheep. Early growth traits were found to be significantly (P≤0.05) affected by ram and ewe in the previous studies carried out by Abbas et al. (2010) in Rahmani and Chios sheep; Esmailizadeh et al. (2011) in Kurdi, Chaal, Afshari and Sanjabi sheep; Màrquez et al. (2012) in Charollais, Suffolk and Texel sheep and Petrović et al. (2013) in Mis and Wurttemberg sheep. In addition, Table 2 shows that all non-genetic effects had significant (P≤0.01) effects on studied criteria. GLM test for these criteria indicated that lamb growth characteristics at pre-weaning were high values for single lambs, lambs born to third parity ewes. Growth advantage of single born might be resulted from its lower competition to milking and supply from the ewe in gestation period than the multiplex (Mousa et al. 2013). This result is in agreement with Roshanfekr et al. (2011, Arabi); Abbasi et al. (2012, Iranian Baluchi, Kurdish); Everett-Hincks et al. (2014, New Zealand breeds) and Simeonov et al. (2015, Blackhead Pleven).

Also, male lambs had higher values for these criteria in comparison with female lambs. The sex difference is consistent as reported by Mohammadi et al. (2010a), Mandal et al. (2012), Mousa et al. (2013) and Everett-Hincks et al. (2014).

Table 1. Descriptive statistics of pre-weaning growth criteria in Romney sheep

Pre-weaning growth criteria	Abbreviations	Mean	SD[#]	C.V[##]
Live body weight at birth (kg)	LBW	5.573	0.8848	15.88
Live body weight at weaning (kg)	LWW	32.48	5.7534	17.71
Kleiber ratio	KR	22.39	2.2246	9.93

[#]standard deviation, [##]coefficient of variability

Table 2. Factors affecting pre-weaning growth criteria in Romney lambs

Factors		№	LBW (kg)		LWW (kg)		KR	
Genetic Factors	Ram (P-Value)	76	≤0.01**		≤0.01**		≤0.01**	
	Ewe (P-Value)	2190	≤0.001***		≤0.01**		≤0.01**	
Non-Genetic Factors			LSM	SD	LSM	SD	LSM	SD
Year	Year-2006	1104	5.502^{d}	0.844	28.551^{f}	4.774	21.252^{f}	1.691
	Year-2007	998	5.166^{e}	0.948	30.332^{e}	5.098	21.445^{e}	1.720
	Year-2008	797	5.579^{c}	0.852	34.354^{c}	5.341	22.837^{b}	1.671
	Year-2009	871	5.863^{a}	0.807	35.990a	4.819	22.170^{c}	1.586
	Year-2010	485	5.851^{a}	0.722	32.503^{d}	4.674	21.752^{d}	1.653
	Year-2012	734	5.695^{b}	0.854	35.163^{b}	5.047	25.734^{a}	1.554
	P-Value		≤0.001***		≤0.01**		≤0.01**	
Parity	Parity-1	3563	5.524^{c}	0.895	32.053^{c}	5.782	22.520^{a}	2.278
	Parity-2	1115	5.628^{b}	0.852	33.375^{b}	5.476	22.078^{b}	1.942
	Parity-3	311	5.934^{a}	0.784	34.322^{a}	5.258	22.380^{a}	2.322
	P-Value		≤0.001***		≤0.01**		≤0.01**	
Sex	Sex-Male	2436	5.702^{a}	0.867	33.781 ^{a}	5.812	22.737^{a}	2.213
	Sex-Female	2553	5.449^{b}	0.884	31.258 ^{b}	5.366	22.102^{b}	2.178
	P-Value		≤0.001***		≤0.001***		≤0.01**	
Birth type	Birth type- 1	816	6.210^{a}	0.674	36.065^{a}	5.243	23.047^{a}	1.968
	Birth type -2	3683	5.527^{b}	0.831	31.952^{b}	5.537	22.246^{b}	2.212
	Birth type -3	490	4.857^{c}	0.902	30.581^{c}	5.558	22.605^{ab}	2.436
	P-Value		≤0.001***		≤0.001***		≤0.01**	
Farm	Farm-1	117	5.204^{e}	1.022	31.081^{f}	5.478	21.874^{de}	1.723
	Farm-2	168	5.607^{ab}	0.871	33.688^{bc}	5.484	21.998 ^{de}	1.640
	Farm-3	112	5.585^{ab}	0.816	30.071^{g}	5.304	21.407^{g}	1.818
	Farm-4	987	5.645^{ab}	0.864	33.449^{bc}	5.383	22.854^{b}	2.271
	Farm-5	152	5.623^{ab}	0.915	33.648^{bc}	5.855	22.418^{c}	1.692
	Farm-6	681	5.701^{a}	0.884	33.997^{b}	5.343	22.806^{b}	2.180
	Farm-7	208	5.638^{ab}	0.805	35.483^{a}	5.228	25.882^{a}	1.564
	Farm-8	428	5.664^{ab}	0.887	32.839^{cde}	5.555	22.722^{b}	2.365
	Farm-9	400	5.508^{bc}	0.935	32.063^{e}	5.685	21.936 ^{de}	1.684
	Farm-10	265	5.522^{bc}	0.852	28.881^{h}	5.294	21.276^{g}	1.913
	Farm-11	342	5.528^{bc}	0.932	32.402^{de}	5.832	22.181^{cd}	2.181
	Farm-12	307	5.411^{cd}	0.839	30.298^{fg}	5.291	21.542^{fg}	1.631
	Farm-13	532	5.344^{d}	0.893	30.496^{fg}	5.383	21.723^{ef}	2.033
	Farm-14	290	5.720^{a}	0.746	33.195^{bcd}	6.237	22.032 ^{de}	1.855
	P-Value		0.045*		0.012**		0.025*	

[#] Means within classification followed by differ letter are differ significantly (Duncan, 0.05)
LSM = least squares mean
* = significant at the 0.05 level
** = significant at the 0.01 level
*** = significant at the 0.001 level

That studied criteria increased from the 1st to 3rd parity and thereafter decreased substantially. A similar significant effect of the parity of ewe on the body weights at early parities was observed by Thiruvenkadan et al. (2011) and Simeonov et al. (2015). Lower values for pre-weaning weights for lambs born to younger ewes may be attributed to the relative competition for nutrients between the still growing ewes and the developing fetus (Thiruvenkadan et al. 2011), also depression in these traits for the lambs born after the fourth parity may be due to the ewe's tooth decay that results in grazing problems followed by decreasing milk production and maternal care for lambs (Mousa et al. 2013).

Average of each criterion fluctuated from year to year. These differences in LBW, LWW and KR among lambs born in different years may be attributed to the differences in environmental conditions especially the rainfall level affecting quantity and quality of grasses. Our results are in agreement with the results obtained, in different sheep breeds, by Roshanfekr et al. (2011), Abbasi et al. (2012) and Simeonov et al. (2015) in Arabi, Iranian Baluchi and Blackhead Pleven, respectively.

The farm had significant effect on the studied criteria, This effect may be due to the differences in management and ram of service, that agreed with the results of Thiruvenkadan et al. (2011), Everett-Hincks et al. (2014) and Simeonov et al. (2015).

Genetic parameters

Variance components

Table (3) shows estimates additive genetic variance (σ^2_a), maternal genetic variance (σ^2_m), permanent environmental variance (σ^2_e), phenotypic variance (σ^2_p) and correlation between additive and maternal additive genetic effects (r_{am}) for different criteria. Estimates of variance components showed that the estimates of σ^2_a were nearly half the estimates of σ^2_m indicating the importance of maternal additive genetic effects on the criteria.

Also, the σ^2_a was found to account for a small proportion and σ^2_e was found to account for a large proportion of the σ^2_p for all the studied criteria. This result reflects the importance of σ^2_e than σ^2_a which is possibly due to uterine capacity, feeding level at late gestation, and maternal behavior of ewe. Reasonably higher maternal additive effects than direct additive effects were observed by El-Awady et al. (2011) and Mousa et al. (2013) for LBW and results obtained by El-Awady et al. (2011) for LWW.

Heritability

Estimation of additive (h^2_a) and maternal (h^2_m) heritabilities and genetic (r_G) and phenotypic (r_p) correlations between the studied criteria are shown in Table (4). These presented results showed that the estimation of h^2_a for LBW and LWW were nearly half the estimates of h^2_m, indicating to the importance of maternal effects in sheep which contribute to the dependence of lambs on their mother`s milk until the time of weaning (Bradford 1972). Morris et al. (1996) worked on New Zealand Romney sheep and reported that heritability estimation for LBW and LWW were 0.29±0.05 and 0.11±0.05, respectively. Everett-Hincks et al. (2014) worked on New Zealand sheep breeds and reported that direct heritability estimates for LBW ranged from 0.126±0.009 to 0.138±0.009 and maternal heritability estimates for LBW ranged from 0.179±0.009 to 0.316±0.01.

Table 3. Variance components for pre-weaning growth criteria in Romney sheep

Criteria	Genetic variance		Phenotypic variance (σ^2_p)	Permanent environmental variance (σ^2_e)
	Direct (σ^2_a)	Maternal (σ^2_m)		
LBW	0.11684	0.34596	0.58784	0.002395
LWW	2.6378	7.1179	17.297	10.1262
KR	0.27565	0.14532	1.9125	0.509339

Table 4. Heritability and additive maternal genetic correlation for pre-weaning growth criteria in Romney sheep

Criteria	Estimates of heritability and additive maternal genetic correlation		
	Direct (h^2_a)	Maternal (h^2_m)	$r_{g(d,m)}$
LBW	0.20 ±0.074	0.59 ±0.219	0.471 ±0.083
LWW	0.15 ±0.042	0.41 ±0.023	0.416 ±0.132
KR	0.14 ±0.052	0.08 ±0.002	0.703 ±0.435

Ghafouri-Kesbi (2013) reported that estimates of direct, maternal heritability, σ^2a, σ^2m, σ^2e and σ^2p for pre-weaning Kleiber Ratio were 0.13±0.03, 0.08±0.03, 0.21, 0.13, 1.21 and 1.66, respectively. In addition, there is a positive genetic correlation between KR with traits related to growth (Abegas et al. 2005, Ghafouri-Kesbi et al. 2011). These findings show that improvements in feed efficiency could be made without negatively affecting body weight or growth rate (Ghafouri-Kesbi 2013). These findings are in accordance with other reports (Abegas et al. 2005; Szwaczkowski et al. 2006; Mohammadi et al. 2010b; Ghafouri-Kesbi et al. 2011; Savar-Sofla et al. 2011; Mokhtari et al. 2012).

Supakorn et al. (2013) reported that in Thailand sheep populations, estimated direct and maternal heritabilities from multivariate analysis of the model for birth weight were 0.32±0.06 and 0.23±0.02, respectively.

Aksoy et al. (2016) reported that in Karayaka lamps, the direct heritability of birth weight was 0.44±0.063. When the maternal genetic effects were considered in models, the direct heritability for birth weight decreased from 0.36 to 0.24. The inclusion of the maternal, genetic, and/or environmental effects into the model resulted in a direct additive variance value varied between 0.07 and 0.08. In model considered maternal genetic and environmental effects, the values for the maternal heritability ranged between 0.15 and 0.22. Furthermore, depending on the model used, direct heritability estimates for WW ranged between 0.40 and 0.27. For weaning weight, model 1 provided a direct heritability value of 0.40±0.066. For weaning weight, the maternal heritability values within the range of 0.04-0.14 were estimated.

Meyer (1992) suggested that models not considering maternal genetic effects could result in substantially higher estimates of σ^2_a and h^2_a. Also, the estimates of h^2_a and h^2_m for LBW were higher than their corresponding estimates for LWW. The decrease in the estimation of both h^2_a and h^2_m by age was in the line with the results of El-Awady et al. (2011) and Mousa et al. (2013). Table 4 shows that estimates of direct and maternal heritability for pre-weaning Kleiber Ratio were 0.14 ±0.052 and 0.08 ±0.002, respectively; which ranged between 0.01-0.15 as reported for different sheep breeds (Mohammadi et al. 2010b; Ghafouri-Kesbi et al. 2011; Savar-Sofla et al. 2011; Mokhtari et al. 2012). Furthermore, Kleiber Ratio estimates of heritability ranged from 0.04 (Arman sheep, Mokhtari et al. 2012) to 0.15 (Sanjabi sheep, Mohammadi et al. 2010b). Estimates of heritability for a criterion may differ between sheep breeds and change slowly over time. Some literature of heritability estimation revealed that in sheep, the Kleiber ratio was a low heritable criterion.

Generally, the obtained results fell in the range reported in the literature for different breeds of sheep maintained at different locations and regions around the world. Range of h^2_a estimates for these criteria in literature varies substantially from 0.004 in Barbary sheep (Bedhiaf et al. 2000) to 0.94 in Hissardale sheep (Chaudhry & Shah 1985) for LBW; from 0.007 in Barbary sheep (Bedhiaf et al. 2000) to 0.81 in Multibreed meat sheep (Lobo et al. 2009) for LWW. Likewise, the estimates of h^2_m ranged from 0.02 in Dorper sheep (Neser et al. 2001) to 0.65 in Sangsari sheep (Miraei-Ashtiani et al. 2007) for LBW and from 0.01 in Ghazel sheep to 0.48 in Dorper sheep (Assan et al. 2011) for LWW and from 0.01 in Romanov sheep (Maria et al. 1993) to 0.07 in crossbreed sheep (Hall et al. 1995). Furthermore, direct estimates of heritability tend to be higher than maternal for early growth traits (Hassen et al. 2003). The range of direct heritability estimates for birth weight from multivariate analysis in the literature varies substantially from 0.04 in Romanov and Kermani breeds (Maria et al. 1993; Rashidi et al. 2008) to 0.46 in Menz breed (Gizaw et al. 2007). Talebi et al. (2007) reported that estimates of heritability for LBW and LWW of Lori-Bakhtiari sheep were 0.30±0.03 and 0.13±0.03 direct and 0.22 and 0.17 maternal, respectively. Consequently, genetic progress is possible for growth traits by selection.

Direct estimates of heritability in sheep breeds for LBW were 0.28±0.04 in Sabi (Matika et al. 2001), 0.06 in Swedish finewool (Näsholm & Danell 1996), 0.13 in Suffolk (Yamaki 1994), 0.14-0.20 in Baluchi (Yazdi et al. 1997), 0.43 in Segurena (Analla et al. 1997) and 0.15±0.05 in Romney (Ibrahim 2015). Furthermore, estimates for LWW were 0.17±0.00 in Sabi (Matika et al. 2001), 0.34 in Suffolk (Yamaki, 1994), 0.15 Swedish finewool (Näsholm & Danell 1996), 0.13-0.19 Baluchi (Yazdi et al. 1997), 0.31 Segurena (Analla et al. 1997) and 0.08±0.03 in Romney (Ibrahim 2015). Gowanea et al. (2015) reported that estimates of direct h^2 were 0.29±0.05, 0.40±0.06, and 0.43±0.06 and the maternal estimates were 0.23±0.04 and 0.15±0.03 for body weights at birth and weaning in Indian Malpura sheep, respectively

Genetic and phenotypic correlations

Table 5 presents the genetic correlations for LBW with both LWW and KR were 0.325 and 0.048, respectively, however, the phenotypic correlations between studied criteria ranged from 0.196 to 0.597 as positive and significant coeffecients.

Table 5. Genetic (above) and phenotypic (below) correlations between pre-weaning growth criteria in Romney sheep

Criteria	LBW	LWW	KR
LBW	-	0.325 (direct) 0.348 (maternal)	0.048 (direct) 0.052 (maternal)
LWW	0.464**	-	0.066 (direct) 0.025 (maternal)
KR	0.196*	0.597**	-

* = Correlation is significant at the 0.05 level ** = Correlation is significant at the 0.01 level

Estimates of genetic correlations between pre-weaning criteria in the current study were positive and moderate. On the other hand, the estimation of genetic correlations for the birth weight ranged from -0.078±0.0084 to -0.127±0.0450 as reported by (Everett-Hincks et al. 2014).

Correlation coefficients were 0.38, and ranged from 0.39 to 0.41 for phenotypic correlation and 0.40, and ranged from 0.40 to 0.81 for genetic correlation in breeds of Suffolk and Baluchi respectively (Yamaki 1994; Yazdi et al. 1997). In general, the estimates of genetic correlations were agree with those reported by El-Awady et al. (2011) in Egyptian Rahmani lambs and lower than those reported by Rashidi et al. (2008); Roshanfekr et al. (2011); Prakash et al. (2012) in Arabi, Kermani and Malpura breeds of sheep, respectively.

Estimated breeding value

Estimated Breeding Value (EBV) are expressed as deviations from population mean and sires were ranked based on their genetic merit. For low heritable below 0.15 criteria, an animal's performance is much less useful for identifying the individuals with the best genes for the trait. For this reason, selection for studied traits should be according to estimates of breeding values. Estimates of maternal heritability were high for pre-weaning criteria as reveal in Table 4. It shows that genes contributing to maternal performance have an equal influence on the early growth of lambs as genes carried by lambs. If maternal genetic effects are not considered, true genetic potential of lambs is masked by maternal performance making it difficult to select superior animals. As a result, maternal effects should be included in the model to obtain accurate estimates of genetic parameters and breeding values, in particular where animals are evaluated for criteria related to early growth.

Table 6 presents the mean, SD, minimum and maximum for direct and maternal estimated breeding values for LBW, LWW and Kr in rams and ewes of Romney sheep. The mean, SD (Min : Max) for Rams were 0.0023±0.1998 (-0.5546 : 0.5017) direct and -0.0039±0.3437 (-0.8632 : 0.9543) maternal EBV for LBW, respectively; were 0.1984±0.7529 (-1.5535 : 3.0056) direct and -0.3262±1.2379 (-4.9417 : 2.5542) maternal EBV for LWW, respectively; and were -0.0226±0.1330 (-0.6333 : 0.2417) direct and -0.0165±0.0983 (-0.4689 : 0.1790) maternal EBV for KR, respectively. The estimated breeding values for rams had a wide range of all pre-weaning criteria, so we may select superior rams and using the selected animals in mating as parents of next generation to make genetic improvement in pre-weaning as a selection objective.

The mean, SD (Min : Max) for ewes were 0.0±0.2307 (-0.6638 : 0.8302) direct and 0.0±0.3970 (-1.4286 : 1.1422) maternal estimated breeding values (EBV) for LBW, respectively; were -0.0069±0.9606 (-2.9963 : 4.5864) direct and 0.0113±1.5793 (-7.5410 : 4.9195) maternal EBV for LWW, respectively; and were 0.0008±0.2314 (-1.6512 : 0.6769) direct and 0.0006±0.1701 (-1.2227 : 0.4919) maternal EBV for KR, respectively. The estimated breeding values for Ewes had a wide range for all pre-weaning criteria, so we may cull lower Ewes in EBV for pre-weaning criteria from next generation to make genetic improvement in pre-weaning as a selection objective.

Jeichitra et al. (2015) reported that the estimates of breeding value (kg) of Mecheri rams for body weights via DFREML as an animal model program ranged from -0.534 to 0.665, -2.592 to 2.195 for birth and three months weights, respectively. For the same traits, estimated breeding value (kg) by Best Linear Unbiased Prediction (BLUP) and Least-squares (LS) methods ranged from -0.199 to 0.228 and -1.195 to 1.133; -1.079 to 0.902 and -4.727 to 3.526, respectively.

Table 6. Estimated breeding values for pre-weaning growth criteria in Romney sheep

Item		LBW		LWW		KR	
		Direct	Maternal	Direct	Maternal	Direct	Maternal
Rams (№=76)	Minimum	-0.5546	-0.8632	-1.5535	-4.9417	-0.6333	-0.4689
	Maximum	0.5017	0.9543	3.0056	2.5542	0.2417	0.1790
	Mean	0.0023	-0.0039	0.1984	-0.3262	-0.0226	-0.0165
	SD	0.1998	0.3437	0.7529	1.2379	0.1330	0.0983
Ewes (№=2190)	Minimum	-0.6638	-1.4286	-2.9963	-7.5410	-1.6512	-1.2227
	Maximum	0.8302	1.1422	4.5864	4.9195	0.6769	0.4919
	Mean	0.0000	0.0000	-0.0069	0.0113	0.0008	0.0006
	SD	0.2307	0.3970	0.9606	1.5793	0.2314	0.1701

CONCLUSION

According to the results, it is concluded that the non-genetic factors have the main source of variation for pre-weaning criteria. Estimation of heritability and variance components of the studied criteria proved the importance of maternal effect. Birth weight, weaning weight and Kleiber ratio have been reported as moderately heritable criteria. Positive genetic correlations among these criteria indicate to possibility of improving these criteria using selection program. Furthermore, Kleiber ratio has been recommended as an indirect selection parameter for feed conversion.

ACKNOWLEDGMENT

The authors wish to thank the Gene Marker Lab., Faculty of Agriculture and Life Science, Lincoln University, New Zealand; for permission to use Romney sheep data giving to Dr A.H.M. Ibrahim while his scientific mission in New Zealand, which funding and supporting via the Partnership and Ownership Initiative, the Egyptian Ministry of Higher Education.

REFERENCES

Abbas SF, Abd-Allah M, Allam FM, Aboul-Ellah AA. 2010. Growth performance of Rahmani and Chios lambs weaned at different ages. Aust J Basic Appl Sci. 4:1583-1589.

Abbasi MA, Abdollahi-Apanahi R, Maghsoudi A, VaezTorshizi R, Nejati-Javaremi A. 2012. Evaluation of models for estimation of genetic parameters and maternal effects for early growth traits of Iranian Baluchi sheep. Small Rumin Res. 104:62-69. doi: 10.1016/j.smallrumres.2011.10.003.

Abegaz S, van Wyk JB, Olivier JJ. 2005. Model comparisons and genetic and environmental parameter estimates of growth and the Kleiber ratio in Horro sheep. S Afr J Anim Sci. 35:30-40.

Aksoy Y, Ulutaş Z, Şen U, Şirin E, Şahin A. 2016. Estimates of genetic parameters for different body weights and muscle and fat depths of Karayaka lambs. Turk J Vet Anim Sci. 40:13-20. doi: 10.3906/vet-1504-16.

Analla M, Munoz-Scrrano A, Serradila JM. 1997. Analysis of the genetic relationship between litter size and weight traits is Segurena sheep. Can J Anim Sci. 77:17-21.

Arthur PF, Renand G, Krauss D. 2001. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livest Prod Sci. 68:131-139. doi: 10.1016/S0301-6226(00)00243-8.

Assan N, Mabuku O, Musasira M. 2011. Estimation of covariance components and genetic parameters for weaning weight in Matebele goat, Sabi, Dorper and Mutton Merino sheep. J Anim Sci Adv. 1:38-46.

Bedhiaf S, Bouix J, Clement V, Bibe B, Francois D. 2000. Choice importance of analysis model for the estimation of genetic parameters of growth of meat sheep in Tunisia. 7èmes Rencontres autour des Recherches sur les Rum. Paris (France): Institut National de la Recherche Agronomique. p. 169-172.

Boldman KG, Kriese LA, Van Vleck LD, Van Tassell CP, Kachman SD. 1995. A manual for use of [MTDFREML] A Set of programs to obtain estimates of variances and covariances. Washington DC (USA): USDA/AES.

Bradford GE. 1972. The role of maternal effects in animal breeding: VII Maternal effects in sheep. J Anim Sci. 35:1324-1334.

Chaudhry MZ, Shah SK. 1985. Heritability and correlation of birth weight, weaning weight and 12 month weight in Lohi, Awassi, Hissardale and Kacchi sheep. Pak Vet J. 5:67-71.

El-Awady HG, Oudah SM, Shalaby NA, El-Arian MN, Metawi HR. 2011. Genetic improvement study on pre-

weaning body weight of Egyptian Rahmani lambs under a pure breeding production system. In: Bernués A, Boutonnet JP, Casasús I, Chentouf M, Gabiña D, Joy M, López-Francos A, Morand-Fehr P, Pacheco F, editors. Economic soc. Options Méditerranéennes Série A Séminaires Méditerranéens. 100:311-316.

Esmailizadeh AK, Miraei-Ashtiani SR, Mokhtari MS, Asadi Fozi M. 2011. Growth performance of crossbred lambs and productivity of Kurdi ewes as affected by the sire breed under extensive production system. J Agric Sci Technol. 13:701-708.

Everett-Hincks JM, Mathias-Davis HC, Greer GJ, Auvray BA, Dodds KG. 2014. Genetic parameters for lamb birth weight, survival and death risk traits. J Anim Sci. 92:2885-2895. doi: 10.2527/jas.2013-7176.

Farokhad ML, Nassiri MTB, Roshanfekr H, Fayazi J, Mamouei M. 2010. Genetic parameters for direct and maternal effects on growth traits of Arman lambs. Res J Biol Sci. 1:71-74. doi: 10.3923/rjbsci.2010.71.74.

Farokhad ML, Roshanfekr H, Amiri S, Mohammadi K, Mirzadeh K. 2011. Genetic trends estimation for some of the growth traits in Arman sheep. J Anim Vet Adv. 10:1801-1803. doi: 10.3923/javaa.2011.1801.1803.

Ghafouri-Kesbi F. 2013. (Co) variance components and genetic parameters for growth rate and Kleiber ratio in fat-tailed Mehraban sheep. Arch Tierz. 56:564-572. doi: 10.7482/0003-9438-56-055.

Ghafouri-Kesbi F, Abbasi MA, Afraz F, Babaei M, Baneh H, Abdollahi Arpanahi R. 2011. Genetic analysis of growth rate and Kleiber ratio in Zandi sheep. Trop Anim Health Prod. 43:1153-1159. doi: 10.1007/s11250-011-9816-2.

Ghafouri-Kesbi F, Eskandarinasab MP. 2008. An evaluation of maternal influences on growth traits, the Zandi sheep breed of Iran as an example. J Anim Feed Sci. 17:519-529. doi: 10.22358/jafs/66679/2008.

Gizaw S, Lemma S, Komen H, Johan AM. 2007. Estimates of genetic parameters and genetic trends for live weight and fleece traits in Menz sheep. Small Rumin Res. 70:145-153. doi: 10.1016/j.smallrumres.2006.02.007.

Gowanea GR, Princea LLL, Lopesb FB, Paswana C, Sharma RC. 2015. Genetic and phenotypic parameter estimates of live weight and daily gain traits in Malpura sheep using Bayesian approach. Small Rumin Res. 128:10-18. doi: 10.1016/j.smallrumres.2015.04.016.

Hall DG, Fogarty NM, Gilmour AR. 1995. Performance of crossbred progeny of Trangie fertility Merino and Booroola Merino rams and Poll Dorset ewes 1 Lamb birth weight, survival and growth. Aust J Experimental Agric. 35:1069-1074.

Hassen Y, Fuerst-Waltl B, SÖlkner J. 2003. Genetic parameter estimates for birth weight, weaning weight and average daily gain in pure and crossbred sheep in Ethiopia J Anim Sci. 120:29-38. doi: 10.1046/j.1439-0388.2003.00361.x.

Ibrahim AHM. 2015. Variability of prion protein (PrP) gene and its association with productive performance in Barki lambs. J Am Sci. 11:89-96.

Jeichitra V, Rajendran R, Karunanithi K, Rahumathulla PS. 2015. Comparison of three methods for estimating breeding values of Mecheri rams for body weights. Indian J Anim Res. 49:161-164.

Kleiber M. 1947. Body size and metabolic rate. Physiol Rev. 27:511-541.

Lobo AMBO, Lobo RNB, Paiva ASR, Oliveira SMPD, Faco O. 2009. Genetic parameters for growth, reproductive and maternal traits in a multi-breed meat sheep population. Genet Mol Biol J. 32:761-770. doi: 10.1590/S1415-47572009005000080.

Mandal A, Dass G, Rout PK. 2012. Genetic analysis of growth and feed conversion efficiency of Muzaffarnagari lambs under intensive feeding system. Int J Livest Prod. 3:47-52. doi: 10.5897/IJLP12.007.

Maria GA, Boldman KG, VanVleck LD. 1993. Estimates of variances due to direct and maternal effects for growth traits of Romanov sheep. J Anim Sci. 71:845-849.

Màrquez GC, Huresign W, Davies MH, Emmans GC, Reehe R, Bunger L, Simm G, Lewis RM. 2012. Index selection in terminal sires improves early lambs growth. J Anim Sci. 90:142-151. doi: 10.2527/jas.2011-4294.

Matika O, van Wyk JB, Erasmus GJ, Baker RL. 2001. Phenotypic and genetic relationships between lamb and ewe traits for the Sabi sheep of Zimbabwe. S Afr J Anim Sci. 313:215-221.

Meyer K. 1992. Variance components due to direct and maternal effects for growth traits of Australian beef cattle. Livest Prod Sci. 31:179-204.

Miraei-Ashtiani SR, Seyedalian SAR, Shahrbabak MM. 2007. Variance components and heritabilities for body weight traits in Sangsari sheep, using univariate and multivariate animal models. Small Rumin Res. 73:109-114. doi: 10.1016/j.smallrumres.2006.12.002.

Mokhtari MS, Shahrebabak MM, Shahrebabk HM, Sadeghi M. 2012. Estimation of (co)variance components and genetic parameters for growth traits in Arman sheep. J Livest Sci Technol. 38-47.

Mohammadi K, Aghaei A, Mizadeh KH, Motaghinia G. 2010a. Investigation of maternal effect on early growth traits in Arabi lambs, using single trait animal models. J Anim Sci Vet Adv. 9:1712-1716. doi: 10.3923/javaa.2010.1712.1716.

Mohammadi Y, Rashidi A, Mokhtari MS, Esmailizadeh AK. 2010b. Quantitative genetic analysis of growth traits and Kleiber ratios in Sanjabi sheep. Small Rumin Res. 93:88-93. doi: 10.1016/j.smallrumres.2010.05.005.

Morris CA, Johnson DL, Sumner RMW, Hight GK, Dobbie JL, Jones KR, Wrigglesworth AL, Hickey SM. 1996. Single trait selection for yearling fleece weight or live weight in Romney sheep correlated responses in live

weight, fleece traits, and ewe reproduction. N Z J Agric Res. 39:95-106. doi: 10.1080/00288233.1996.9513166.

Morris CA, Hickey SM, Clarke JN. 2000. Genetic and environmental factors affecting lamb survival at birth and through to weaning. N Z J Agric Res. 43:515-524. doi: 10.1080/00288233.2000.9513448.

Mousa E, Mona A. Osman, EL-Saied UM. 2006. Genetic parameters for body weight of Egyptain Farafra lambs with random regression model. Egypt J Anim Prod. 43:57-69.

Mousa E, Monzaly H, Shaat I, Ashmawy. 2013. Factors affecting birth and weaning weights of native Farafra lambs in Upper Egypt. Egypt J Sheep Goat Sci. 8:1-10.

Näsholm A, Danell. 1996. Genetic relationships of lamb weight, maternal ability, and mature ewe weight in Swedish finewool sheep. J Anim Sci. 74:329-339.

Neser FWC, Erasmus GJ, VanWyk JB. 2001. Genetic parameter estimates for pre-weaning weight traits in Dorper sheep. Small Rumin Res. 40:197-202. doi: 10.1016/S0921-4488(01)00172-9.

Petrović V, Petrović MP, Ilić, Z Petrović MM, Milošević B, Ružić Muslić D, Maksimović N. 2013. Effect of genotype, sire, sex, gestation length on birth weight of lambs. Biotechnol Anim Husb. 29:685-693. doi: 10.2298/BAH1304685C.

Prakash V, Prince LLL, Gowane GR, Arora AL. 2012. The estimation of (co)variance components and genetic parameters for growth traits and Kleiber ratios in Malpura sheep of India. Small Rumin Res. 108:54-58. doi: 10.1016/j.smallrumres.2012.07.018.

Rashidi A, Mokhtari MS, Safi JM, Mohammad AMR. 2008. Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. Small Rumin Res. 74:165-171. doi: 10.1016/j.smallrumres.2007.06.004.

Roshanfekr H, Mamouei M, Mohammadi K, Rahmatnejad E. 2011. Estimation of genetic and environmental parameters affected pre-weaning traits of Arabi lambs. J Anim Vet Adv. 10:1239-1243. doi: 10.3923/javaa.2011.1239.1243.

[SAS] Statistics Analysis System. 2002. Statistics Analysis System: User's guide (Ver 9). North Carolina (US): SAS Institute Inc.

Savar-Sofla S, Nejati-Javaremi A, Abbasi MA, Vaez-Torshizi R, Chamani M. 2011. Investigation on direct and maternal effects on growth traits and the Kleiber Ratio in Moghani Sheep. World Appl Sci J. 14:1313-1319.

Simeonov MS, Harmon DL, Nedelkov KV. 2015. Non-genetic factors affecting birth weight in the lambs of Blackheads Pleven breed. J Anim Sci Adv. 5:1208-1217. doi: 10.5455/jasa.20150326081131.

Supakorn C, Pralomkarn W, Anothaisinthawee S. 2013. Estimation of genetic parameters and genetic trends for weight and body measurements at birth in sheep populations in Thailand. Songklanakarin J Sci Technol. 35:1-10.

Szwaczkowski T, Wójtowski J, Stanisławska E, Gut A. 2006. Estimates of maternal genetic and permanent environmental effects in sheep. Arch Tierz. 49:186-192.

Talebi MA, Miraei-Ashtiani SR, Nejati-Javaremi A, Moradi-Shahrbabak M. 2007. Phenotypic and genetic characteristics of growth and carcass traits of Lori-Bakhtiari sheep. Biotechnol Anim Husbandry. 23:357-363. doi: 10.2298/BAH0701357T.

Thiruvenkadan AK, Karunanithi K, Muralidharan J, Narendra BR. 2011. Genetic analysis of pre-weaning and post-weaning growth traits of Mecheri sheep under dry land farming conditions. Asian-Australas J Anim Sci. 24:1041-1047. doi: 10.5713/ajas.2011.10361.

Wolf JB, Brodie ED, Cheverud JM, Moore AJ, Wade MJ. 1998. Evolutionary consequences of indirect genetic effects. Trends Ecol Evol. 13:64-69. doi: 10.1016/S0169-5347(97)01233-0.

Yamaki K. 1994. Growth of lambs and genetic parameters in four breeds. Proc 5th World Congr Gen Appl Livest Prod. 18:135-138.

Yazdi MH, Engström G, Nasholm A, Johansson K, Jorjani H, Liljedahl LE. 1997. Genetic parameters for lamb weight at different ages and wool production in Baluchi sheep. J Anim Sci. 65:224-255.

In Vitro Rumen Fermentation Characteristics and Microbes of Thin Tail Sheep Given Sweet Potato Biomass

Sudarman A[1], Hayashida M[2], Miralestari M[1]

[1]*Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor 16680, Indonesia*
[2]*Department of Bioproduction Technology, Junior College Tokyo University of Agriculture, Japan*
E-mail: a_sudarman@yahoo.com

(received 28-04-2016; revised 17-05-2016; accepted 09-06-2016)

ABSTRAK

Sudarman A, Hayashida M, Miralestari M. 2016. Karakteristik rumen fermentasi secara *in vitro* dan mikroba domba ekor tipis yang diberikan biomassa ubi jalar. JITV 21(2): 83-87. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1355

Tanaman ubi jalar (*Ipomoea batatas* L) diproduksi di Indonesia dalam jumlah besar. Daun dan batang ubi jalar dapat digunakan sebagai sumber protein dan umbinya dapat digunakan sebagai sumber energi. Penelitian ini bertujuan untuk mengkaji pengaruh pemberian biomasa ubi jalar pada karakteristik fermentasi rumen, kecernaan bahan kering dan bahan organic, serta populasi mikroba rumen domba ekor tipis. Penelitian ini menggunakan rancangan acak kelompok dengan menerapkan empat perlakuan pakan, yaitu, T0 (100% rumput gajah), T1 (70% dari rumput gajah + 30% konsentrat), T2 (50% dari rumput gajah + 50% daun ubi jalar), dan T3 (70% daun ubi jalar + 30% umbi ubi jalar) dengan tiga ulangan. Sampel cairan rumen diambil dengan metode *stomach tube* dengan menggunakan pompa vakum. Hasil penelitian menunjukkan bahwa perlakuan T3 memiliki kecernaan bahan kering dan bahan organik, konsentrasi NH3 dan VFA, dan populasi bakteri rumen lebih tinggi (P<0,05) daripada perlakuan T0 dan T1 tetapi tidak berbeda dengan perlakuan T2. Populasi protozoa dan pH rumen tidak berbeda antar perlakuan. Dapat disimpulkan bahwa penggunaan biomassa ubi jalar dapat meningkatkan kualitas fermentasi rumen domba.

Kata Kunci: Rumen, Kecernaan, Fermentasi, Domba, Biomassa Ubi Jalar

ABSTRACT

Sudarman A, Hayashida M, Miralestari M. 2016. *In vitro* rumen fermentation characteristics and microbes of thin tail sheep given sweet potato biomass. JITV 21(2): 83-87. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1355

Sweet potato plant (*Ipomoea batatas* L) is produced in Indonesia in large quantities. Sweet potato leaves and stems can be used as a source of protein and the tuber can be used as an energy source. *This* study was aimed to analyze the effect of feeding of sweet potato biomass on the rumen fermentation characteristics, digestibility of dry matter and organic matter as well as rumen microbial population of thin tail sheep. This study used a randomized block design by applying four feed treatments, i.e., T0 (100% Napier grass), T1 (70% of Napier grass + 30% concentrate), T2 (50% of Napier grass + 50% sweet potato leaves), and T3 (70% sweet potato leaves + 30% sweet potato tuber) with three replicates. Rumen fluid samples were taken with stomach tube method using a vacuum pump. Results showed that the T3 diet has higher (P<0.05) digestibility of dry matter and organic matter, concentration of NH_3 and VFA, and rumen bacterial population than those of T0 and T1diets but similar to that of T2 diet. Rumen pH and protozoa population was not different among the treatments. It can be concluded that the use of sweet potato biomass can improve the quality of rumen fermentation of sheep.

Key Words: Rumen, Digestibility, Fermentation, Sheep, Sweet Potato Biomass

INTRODUCTION

Low quality and fluctuated availability of feed may lead to low productivity of sheep. Most small holder farmers feed their sheep only by field grass regardless adequacy of the nutrients and nutrition required by sheep.

By-product of agriculture or plantations overflowing around the sheep farms may be used as an alternative good quality and cheap price feed. In Bogor, for example, are widely grown sweet potatoes producing waste that can be utilized by small holder farmers to feed their sheep. Sweet potato is widely grown by farmers because its relatively easy processing, have relatively short harvest period and resistant to drought.

In 2014, production of sweet potato in Indonesia amounted to 2,382,025 tonnes, while in West Java amounted to 471,737 tonnes and in Bogor amounted to 82,935 tonnes (CSA 2015). Sweet potato vines may be used as protein feed source containing 19.38% crude protein and 3720 Kcal/kg energy (Kebede et al. 2008), whereas the tubers can be used as an energy source with

energy content of 4156 Kcal/kg and crude protein content of 5.5% (Heuzé et al. 2015).

Results of previous studies show that the addition of sweet potato crops in napier grass-based diets may improve rate of degradation by rumen microbes and rumen fermentation which increases feed intake and animal productivity (Kariuki et al. 2001). This in vitro study was aimed to analyze effect of giving biomass sweet potato (*Ipomoea batatas* L.) on rumen fermentation characteristics and microbial rumen of sheep.

MATERIALS AND METHODS

Napier grass, sweet potato leaves, and sweet potato tubers flour were previously prepared by drying them for 2-3 days and then finely grounded. Concentrate were made by mixing coconut meal 30%, wheat pollard 15.29%, peanut meal 25%, cassava waste flour 27.08%, $CaCO_3$ 1.93%, salt 0.2%, and premix 0.5%. Treatment diets were T0: 100% grass, T1 = 70% of Napier grass + 30% concentrate, T2 = 50% Napier grass + 50% potato leaves, T3 = 70% leaf + 30% potato tubers. Nutrient content and composition of the treatments used are presented in Table 1.

Approximately 15-20 ml of rumen liquor were collected from three sheep before morning meal using a stomach tube with vaccum pump. Samples were filtered and analysed for protozoa and bacteria (Ogimoto & Imai 1981), ammonia concentration (Conway 1962), total volatile fatty acids (VFAs) using steam distillation method (GLP 1966), dry matter and organic matter digestibility (Tilley & Terry 1963), and rumen pH. Experimental design was based on a randomized complete block design using four treatments as describes above with three blocks for each treatment. The blocks were based on three sheep that had been taken their rumen fluid. Data were analyzed using analysis of variance (ANOVA) (Steel & Torrie 1993). Any significant differences ($P<0.05$) of mean were further tested using Duncan's multiple range test. All statistical analysis were performed using SPSS software version 16.0.

RESULTS AND DISCUSSION

Rumen fermentation characteristics

Results on rumen fermentation characteristic variables were presented in Table 2. Those three treatments had no significant ($P>0.05$) effect on rumen pH. Rumen pH value has an important role in supporting rumen microbial growth. Proper rumen microbial growth leads to normal fermentation process producing VFA and NH_3 (Uhi et al. 2006). Rumen pH values in this study were in normal range around neutral, i.e., 6.93-7.00.

Those treatments significantly ($P<0.05$) affected VFA concentration. VFA value in treatment T3 (70% leaves and stems of sweet potatoes + 30% sweet potato tubers) did not differ from T2 treatment but was higher than the T1 and T0 treatment (Table 2). This is because of T3 treatment contained the highest NFE in the ration (58.91%) derived from feed energy source use, namely, sweet potato tubers. On the other hand due to the relatively low content of NFE in the ration (47.42%), T0 treatment had the lowest VFA concentration compared with other treatments (Table 2). NFE is an easily digestible carbohydrate that can be used as an energy source for livestock (Farida & Ridwan 2011). Easily digestible carbohydrates are in group of

Table 1. Chemical composition (% DM basis) of experimental diets

Nutrient	Treatment diets*			
	T0	T1	T2	T3
Dry matter (DM)	21.56	42.017	18.025	18.444
Crude protein (CP)	7.97	8.879	13.195	13.662
Ether extract (EE)	5.1	5.755	6.345	5.571
Crude fibre (CF)	26.9	22.001	21.28	11.841
Nitrogen free extract (NFE)	47.42	50.24	46.705	58.911
Ash	12.61	13.123	12.72	10.358
TDN**	54.97	58.207	62.29	74.161

Results of laboratory analyzes of Research Center for Biological Resources and Biotechnology of IPB (2015)
**Result of the calculation according to Hartadi et al. (1997)
*T0 = 100% Napier grass
T1 = 70% Napier grass + 30% concentrate
T2 = 50% Napier grass + 50% sweet potato leaves
T3 = 70% sweet potato leaves + 30% sweat potato tuber

Table 2. Effect of treatment on the rumen fermentation characteristics

Parameters	Treatment diets*			
	T0	T1	T2	T3
pH	6.97±0.06	7.00±0.00	6.93±0.06	7.00±0.00
VFA (mM)	99.94±6.46^{c}	107.42±6.90^{bc}	116.39±11.69^{ab}	125.27±6.77^{a}
NH_3 (mM)	6.89±0.44^{c}	7.80±1.30^{bc}	9.01±1.53^{ab}	10.41±1.42^{a}
IVDMD (%)	45.23±3.92^{c}	47.90±1.88^{c}	62.50±1.39^{b}	72.29±4.60^{a}
IVOMD (%)	40.25±5.95^{c}	45.81±1.69^{c}	61.55±1.88^{b}	72.10±5.58^{a}

*T0 = 100% Napier grass
T1 = 70% Napier grass + 30% concentrate
T2 = 50% Napier grass + 50% sweet potato leaves
T3 = 70% sweet potato leaves + 30% sweat potato tuber
Different superscript within the same row shows significant (P<0.05) difference

polysaccharide starch. Starch in rumen will be hydrolyzed into monosaccharide by rumen microbial enzymes and further fermented into VFA such as acetate, propionate, and butyrate, and gases of CH_4 and CO_2. Total VFA concentration in this study was slightly lower than that of the finding of Puastuti et al. (2012) that was 122.5-190.3 mM. VFA production of a feedstuff reflects its fermentability level. VFA produced was higher along with higher fermentability level of a feedstuff.

Those three treatment also had a significant effect (P<0.05) on the concentration of NH_3. Concentration of NH_3 result had the same pattern as the VFA result. Concentration of NH_3 in treatment T3 (70% leaves and stems of sweet potatoes + 30% sweet potato tubers) did not differ from that of T2, but was higher than those of T1 and T0 treatments (Table 2). This figure caused by protein content in rations T3 was the highest (13.66%) compared to other treatments (Table 1). Feed protein undergoes hydrolyisis into oligopeptide by proteolytic enzymes. Oligopeptides were then undergo deamination and converted into an amino acid producing NH_3. High crude protein content in feed will produce high NH_3. Concentration of ammonia in the rumen depends on protein content of feed. Quality of protein in the diet may also affect the concentration of ammonia. Large quantities of low quality protein would not be beneficial, because most of the protein would be degraded by rumen microbes into ammonia. Excess of ammonia will be discarded through urine causing low efficiency of protein utilization. T0 treatment contained the lowest crude protein (7.97%), therefore produced the lowest NH_3 concentration compared to other treatments. NH_3 concentration of all treatments in this study were comparable with the results of Puastuti et al. (2012) showing that NH_3 concentration of sheep fed napier grass and supplemented with rumen undegradable protein ranged from 7.4 to 9.4 mM.

Digestibility of dry matter and organic matter

Those three treatments significantly (P<0.01) affected digestibility of dry matter and organic matter. Digestibility of dry matter and organic matter of T3 treatment (70% leaves and stems of sweet potatoes + 30% sweet potato tubers) was the highest compared to those T0, T1 and T2 treatment. Digestibility value of feeds was in contrast to their crude fibre content. The lower crude fiber content, the higher digestibility value of feed. Crude fibre content of T3 treatment was the lowest followed by treatment of T2, T1, and T0, i.e., 11.8%, 21.3%, 22.0% and 26.9%, respectively. Results were in line with the results of Aregheore (2004) showing that sweet potato had digestibility more than 62%.

Digestibility value of T1 treatment was not significantly different from the digestibility value of T0 treatment (Table 2). This clearly shows that the addition of 30% concentrate on the treatment T1 could not increase digestibility value of feed. Digestibility value in T0 treatment (fed only grass) was the lowest compared to that of other treatments (Table 2). It was caused by the high crude fiber content of elephant grass in the T0 ration, i.e., 26.9%, where the crude fiber is a component that is difficult to be digested.

Rumen microbial population

Population of ruminal bacteria and protozoa in this study were presented in Table 3. High number of bacterial populations on T3 treatment was followed by increase of concentration of VFA and NH_3. This indicates that bacteria on the T3 treatment were capable in utilizing VFA and ammonia from feed degradation for their growth. In turn, it produces more bacterial population that may accelerate fermentation process of feed. So that, VFA and NH3 results will also increase.

Table 3. Effect of treatment on the rumen microbial population

Parameters	Treatment diets*			
	T0	T1	T2	T3
Bacteria (Log CFU ml^{-1})	8.29 ± 0.02^{c}	8.35 ± 0.01^{b}	8.37 ± 0.04^{ab}	8.42 ± 0.02^{a}
Protozoa (Log cell ml^{-1})	6.39±0.09	6.36±0.09	6.35±0.10	6.30±0.09

*T0 = 100% Napier grass
T1 = 70% Napier grass + 30% concentrate
T2 = 50% Napier grass + 50% sweet potato leaves
T3 = 70% sweet potato leaves + 30% sweat potato tuber
Different superscript within the same row shows significant (P<0.05) difference

The results was supported by Siwaporn et al. (2010) who reported that in cattle producing high VFA and in buffalo producing high N-NH_3, the ruminal bacteria population was high. Increase of VFA amount may be caused by the presence of amylolytic and cellulolytic bacterial population that is capable to degrade fiber and starch in feed into VFA. Bacterial population of T0 (100% grass) was the lowest compared to that of the other treatments. This may be due to the protozoa populations of T0 treatment tend to be highest. It is well known that high population of protozoa may prey on population of cellulolytic and amylolytic bacteria. As a consequent, the VFA produced is low. The present results proved that low population of bacteria of the T0 treatment was accompanied by the low concentration of the VFA. Protozoa population in T3 treatment tended to be lower, so that T3 treatment bacterial population increased. In the T3 treatment, sweet potato leaves with its saponin content (Anthoney & Omwenga 2014) was able to lysis protozoa cell causing them to death, but saponin had no effect on bacterial cells.

Total bacteria in this experiment (19.50×10^7/ml - 26.30×10^7/ml) were lower that reported by Singh & Kundu (2011) (8.53×10^8/ml - 16.05×10^8/ml) for the sheep fed by *Dicanthium annulatum* grass based diet and supplemented with *Leucaena leucocephala* and *Hardwickia binata*. This discrepancy probably due to that many of bacteria in this experiment were eaten by protozoa, as shown by the number of protozoa in this study was higher than the protozoa reported by Singh & Kundu (2011).

The treatments did not significantly (P>0.05) affect protozoa population (Table 3). T2 and T3 treatments containing sweet potato leaves tended to have lower protozoa population than T0 and T1. There was a tendency showing that with increasing level of sweet potato leves, the protozoa population increased. This may indicate that sweet potato leaves have an anti protozoa agent. Eventhough the effect was not so strong. Sweet potato plant is known to contain anti-nutrients of saponin (Anthoney & Omwenga 2014). Saponin usually used as defaunating agents being capable to lysis protozoa cell (Wang et al. 2011). Thus higher sweet potato leaves given produces higher saponin content in the feed that is able to lysis protozoa cell in greater numbers. Sweet potato leaves contain saponin (Pochapski et al. 2011) as defaunating agent being capable in reducing protozoa population. Protozoa population of the T0 (100% elephant grass) and T1 (70% of elephant grass + 30% concentrate) tended to be higher than that of T2 and T3 treatments. T0 and T1 treatmnets did not use sweet potato leaves, so it did not contain saponin that may reduce population of protozoa. Lower rumen protozoa populations of sheep were reported by Singh & Kundu (2011) as 5.34 and 3.95×10^5/ml in rumen of sheep fed by *Dicanthium annulatum* grass based diet and supplemented with *Leucaena leucocephala* and *Hardwickia binata* in 75:25 ratio, respectively. Lower protozoa population of Singh & Kundu (2011) than that in this study probably due to that *Leucaena leucocephala* and *Hardwickia binata* had higher secondary compound (condensed tannin and saponin) that capable in reducing protozoa population.

CONCLUSION

Use of sweet potato biomass may improve quality of ruminal fermentation of sheep. Providing of 30% tubers and 70% leaves (T3 diet) of sweet potato afeected dry and organic matters digestibility concentration of NH_3 and VFA as well as bacterial population were higher than other treatments. Moreover, it had no siginificant effect on protozoa population.

ACKNOWLEDGEMENT

Authors wish to thank "International Cooperative Research Project (TUA-IPB-MSU)" of Tokyo University of Agriculture for the financial support.

REFERENCES

Anthoney ST, Omwenga J. 2014. Analysis of phytochemical composition of white and purple sweet potato (*Ipomoea batatas* L.). J Adv Plant Res. 1:19-22.

Aregheore EM. 2004. Nutritive value of sweet potato (*Ipomoea batatas* L.) forage as goat feed: voluntary intake, growth and digestibility of mixed rations of sweet potato and batiki grass (*Ischaemum aristatum* var. *indicum*). Small Rumin Res. 51:235-241.

[CSA] Central Statistic Agency. 2015. Statistik Indonesia 2015. Jakarta (Indones): Central Statistic Agency.

Conway EJ. 1962. Micro-diffusion analysis and volumetric error. 5th ed. London (UK): Crosby Lockwood & Sons Ltd.

Farida WR, Ridwan R. 2011. Giving of formulated pellet on Javan Porcupine (*Hystrix javanica* F. Cuvier, 1823): Effects on feed intake, feed conversion, and digestibility in pre-domestication condition. J Bio Indones. 7:157-170.

[GLP] General Laboratory Procedures. 1966. Department of Dairy Science. Madison (USA): University of Wisconsin.

Hartadi HS, Reksohadiprodjo, Tillman AD. 1997. Tabel komposisi pakan untuk Indonesia. Yogyakarta (Indonesia): Gadjah Mada University Press.

Heuzé V, Tran G, Hassoun P, Renaudeau D, Bastianelli D. 2015. Sweet potato (*Ipomoea batatas*) tubers. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. [accessed September 7th 2016]. http://www.feedipedia.org/node/745.

Kariuki JN, Tamminga S, Gachuiri CK, Gitau GH, Muia JMK. 2001. Intake and rumen degradation in cattle fed Napier grass (*Pennisetum purpureum*) supplemented with various levels of *Desmodium intortum* and *Ipomoea batatas* L. vines. J Anim Sci. 31:149-157.

Kebede T, Lemma T, Tadesse E, Guru M. 2008. Effect of level of substitution of sweet potato (*Ipomea batatas* L) vines for concentrate on body weight gain and carcass characteristics of browsing Arsi-Bale goats. J Cell Anim Bio. 2:036-042.

Pochapski MT, Fosquiera EC, Esmerino LA, dos Santos EB, Farago PV, Santos FA, Groppo FC. 2011. Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from *Ipomoea batatas* (L.) Lam. Pharmacogn Mag. 7:165-170. doi: 10.4103/0973-1296.80682.

Siwaporn P, Wanapat M, Wachirapakorn C, Nontaso N. 2010. The effect of roughage and urea solution infusion levels on ruminal NH3-N concentration and nutrient digestibility in beef cattle and swamp buffaloes. Silpakorn U Sci Technol J. 4:47-55.

Ogimoto K, Imai S. 1981. Atlas of rumen microbiology. Tokyo (Japan): Japan Scientific Societes Press.

Puastuti W, Yulistiani D, Mathius I-W. 2012. Ruminal fermentation response and nitrogen retention from sheep fed rumen undegradable protein. Indones J Anim Vet Sci. 17:67-72.

Singh S, Kundu SS. 2011. Comparative rumen microbial population in sheep fed *Dicantium annulatum* grass supplemented with *Leucaena leucocephala* and *Hardwickia binate* tree leaves. Livest Res Rural Develop. Vol. 23. Article #7. http://www.lrrd.org/lrrd23/1/ sing 23007.htm.

Steel RGD, Torrie JH. 1993. Prinsip dan prosedur statistika. Edisi Ke-3. Terjemahan. Jakarta (Indones): PT Gramedia.

Tilley JMA, Terry RA. 1963. A two-stage technique for the in vitro digestion of forage crops. J Br Grassl Soc. 18:104-111.

Uhi HT, Parakkasi A, Haryanto B. 2006. Pengaruh suplemen katalitik terhadap karakteristik dan populasi mikroba rumen domba. J Anim Sci Technol. 29:20-26.

Wang JK, Ye JA, Jian-Xin L. 2011. Effects of tea saponins on rumen microbiota, rumen fermentation, methane production and growth performance. Trop Anim Health Prod. 44:697-706.

Effect of Different Protein and Energy Levels in Concentrate Diets on Nutrient Intake and Milk Yield of Saanen x Etawah Grade Goats

Supriyati, Krisnan R, Budiarsana IGM, Praharani L

Indonesian Research Institute for Animal Production, PO Box 221, Bogor 16002
E-mail: skompiang@yahoo.co.id

(received 11-05-2016; revised 24-05-2016; accepted 21-06-2016)

ABSTRAK

Supriyati, Krisnan R, Budiarsana IGM, Praharani L. 2016. Pengaruh dari perbedaan tingkatan protein dan energi pakan konsentrat terhadap asupan nutrisi dan produksi susu pada kambing Saanen x Etawah silangan. JITV 21(2): 88-95. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1356

Kambing perah turut berkontribusi pada ketahanan pangan dan nutrisi. Akan tetapi, informasi terkait konsumsi nutrisi dan produksi susu (termasuk komposisinya) kambing persilangan Saanen x Etawah (SAPERA) masih terbatas. Penelitian ini bertujuan untuk mengevaluasi asupan nutrisi, produksi susu dan komposisi kandungan nutrisi kambing SAPERA yang sedang menyusui yang diberi pakan dengan tingkat energi dan protein konsentrat yang berbeda. Tiga puluh kambing SAPERA multipara dianalisis menggunakan rancangan acak kelompok dengan tiga perlakuan (R1, R2, dan R3) dan 10 pengulangan selama 12 minggu laktasi. Pakan konsentrat yang diformulasikan mengandung 18% PK dan 72% TDN (R1), 17% PK dan 75% TDN (R2), dan 16% PK dan 78% TDN (R3). Domba betina dipelihara dalam kandang individu dan diberikan pakan basal (cacahan rumput raja segar secara ad libitum sebanyak 500 g dari campuran pakan hijauan) dan 1 kg konsentrat perlakuan. Hasil penelitian menunjukkan bahwa perlakuan (R1, R2, dan R3) memiliki pengaruh nyata ($P<0.05$) terhadap asupan PK, PT, Ca, P dan FCR tetapi tidak berpengaruh nyata ($P>0.05$) terhadap asupan BK dan TDN. Perbedaan yang tidak nyata ditemukan pada produksi dan komposisi susu antar perlakuan. Berdasarkan hasil penelitian, dapat disimpulkan bahwa pakan terbaik untuk kambing SAPERA menyusui adalah campuran dari rumput cacah, campuram hijauan dan konsentrat (16% PK dan 78% TDN) dengan 160g/kg PK dan 750 g/kg TDN dari total BK yang memproduksi susu sebanyak 1.55 kg/hari dengan kandungan 90 g/hari lemak, 43 g/hari protein dan 75 g/hari laktosa.

Kata Kunci: Energi, Protein, Kambing Persilangan Saanen x Etawah, Laktasi, Produksi Susu

ABSTRACT

Supriyati, Krisnan R, Budiarsana IGM, Praharani L. 2016. Effect of different protein and energy levels in concentrate diets on nutrient intake and milk yield of Saanen x Etawah Grade goats. JITV 21(2): 88-95. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1356

Dairy goat contributes to food and nutrition security. However, information on nutrient consumption and milk yield, as well as milk composition of Saanen x Etawah (SAPERA) grade goat is limited. This experiment was done to evaluated nutrient intake, milk yield and its composition of lactating SAPERA goats fed with different levels of dietary energy and protein in concentrate diet. Thirty multiparous SAPERA goats were used in a randomized block design with three treatments (R1, R2 and R3) and ten replications for 12 weeks of lactation. The concentrate diets were formulated to contain: 18% CP and 72% TDN (R1), 17% CP and 75% TDN (R2), 16% CP and 78% TDN (R3). Those does were penned individually, and fed by basal diet (fresh chopped King Grass ad libitum, 500 g of fresh mixed forages) and 1 kg of experimental concentrate. Results showed that the treatments had significant ($P<0.05$) effects on CP, DIP, Ca, P intakes and FCR but had no significant ($P>0.05$) effects on DM and TDN intake. No significant differences were found in milk yield and milk composition between treatments. In conclusion, this trial suggested that the best feed for lactating SAPERA goats was the mixture of chopped grasses, mixed forages and concentrate diets (16% CP and 78% TDN) with 160 g/kg CP and 750 g/kg TDN of the total DM, produced a milk of 1.55 kg/d with 90 g/day of milk fat, 43 g/day of milk protein and 75 g/day of milk lactose.

Key Words: Energy, Protein, Saanen X Etawah Grade Goat, Lactation, Milk Yield

INTRODUCTION

Population of goat in Indonesia was around 18.88 million heads in 2015 (DGLAH 2015) used for milk and meat production. This Saanen breed was introduced in to breeding program of the Indonesian Research Institute for Animal Production to improve quality and quantity of goat milk yield. This Saanen breed often produced triplets (Mellado et al. 2011) and higher milk yield compared to Etawah Grade (Praharani 2014) and Angora goats (Anwar et al. 2015). Therefore, Saanen genetics were used to produce a new goat breed with

higher milk yield and adapted well to Indonesian environmental conditions. Saanen goats were crossed with Etawah goats to produce crossbred Saanen and Etawah grade goats, named as SAPERA.

Information on feed intake and nutrient utilization of these SAPERA goats under traditional or intensive production systems are infrequent in Indonesia. Goat feeding involves combining various feedstuffs into an acceptable and palatable ration to meet nutrient requirements. These requirements vary depending on the stage of growth, gestation and lactation. The considered nutrients in diet formulation are energy, protein, minerals, vitamins and water. The balance of nutrients will determine performance of a dairy goat. Lactating doe requires high level of energy, protein, and water for milk yield. Basal diets of dairy goats were often supplemented with concentrate to meet their requirements.

Nutritional requirements of energy and protein of goats have been reported and reviewed by some previous researchers. Krishnamoorthy & Moran (2011) reviewed that the nutritional requirement of goats in the tropic could refer to as recommended by the Nutrient Requirement Council (NRC). Energy required by female Etawah grade goat was 1.1 times NRC (Supriyati et al. 2014a) and for female Anglo Nubian goat was 1.2 times NRC (Supriyati et al. 2014b). Martínez-Marín et al. (2011) reported that intake of metabolism energy (ME) was 5.4% greater than that recommended by the NRC for young female Murciano-Granadina dairy goats. Park et al. (2010) suggested that minimum dietary level of protein and energy was 15% CP and 60% TDN in mid lactation for Saanen dairy goats.

This study was aimed to evaluate effect of different level protein and energy in concentrate diets on nutrient intake, milk yield and milk composition of SAPERA goats during the first 12 weeks lactation.

MATERIALS AND METHODS

Animal and feeding trial

Thirty multiparous of SAPERA goats, around 3-4 years with an average body weight of 40.75±3.35 kg, were used in this trial. Animals were grouped into three concentrate diets treatments. Those concentrate diets were formulated at different crude protein (CP) and total digestible nutrients (TDN) levels, i.e. R1 = 18%CP and 72%TDN, R2 = 17% CP and 75%TDN and R3 = 16%CP and 78%TDN on dry matter (DM) basis. Animals were offered chopped fresh King grass *ad libitum*, 500 g of fresh mixed forages and 1 kg of concentrate diet as feed during the first 12 weeks of the lactation period. Table 1 shows the chemical composition of feed. The experimental design applied was completely randomized in three treatments and ten replications. Each animal was housed in individual cage. Those cages had metal wire galvanized floors and attached to each cage was a secured woody container for feed. Water was provided through a nipple in each cage. Feed intakes were measured daily.

Parameters observed were nutrient intake of DM, CP, digestible intake protein (DIP), TDN, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca) and phosphorus (P). DM, CP, NDF, ADF, Ca and P contents of the grass, mixed forage and concentrate diets were analyzed according to the AOAC method (AOAC 2012) modified in ours laboratory. Gross energy values were determined by bomb calorimeter (Adiabatic Bomb, Parr Instrument Co), and these values were used for TDN calculation as described by NRC (1981). Percentage of total digestible of nutrient (TDN) = Kcal/kg metabolism of energy (ME) divided by 0.0361, where ME is equal with 0.62 Kcal/kg of Gross Energy (NRC 1981) and 0.0361 is the conversion factor of ME to TDN as described by Langston University's ME calculator.

Table 1 Chemical composition of feed on DM basic

Variables (%)	Grass	Mixed forages	Concentrate diets		
			R1 18% CP, 72% TDN	R2 (17% CP, 75%TDN	R3 (16%CP, 78%TDN)
Crude protein	9.09	20.56	18.23	17.32	16.32
Total digestible nutrient	67.20	74.44	72.13	74.96	77.48
Neutral detergent fiber	63.65	56.24	41.32	39.56	38.66
Acid detergent fiber	48.27	50.71	21.40	22.37	21.58
Calcium	0.34	1.39	1.02	1.19	1.13
Phosphorus	0.29	0.16	0.94	0.96	0.88

At the end of the experiment, digestible intake protein (DIP) was measured using total collection technique in metabolism cages. Four animals of each treatment from similar experimental goats were pleaced in individual metabolism cages. These animals were allowed ten days to adjust to the feed, followed by seven days collection. Feed intake, refusals and fecal output were recorded and kept, and a sub sample of each (10% of daily output in case of feces) was retained for analysis. Samples were then dried, grounded, and analyzed for protein.

Digestibility of protein intake (DIP) was calculated as follows:

$$\text{DIP (\%)} = \frac{\text{Protein intake} - \text{Protein in feces}}{\text{Protein intake}} \times 100$$

Milk yield and samples

Goats were milked by hand in the morning and evening. Individual morning and evening milk yields were recorded daily for each goat. The 4% fat corrected milk (FCM) for each goat was calculated from milk yield and percentage of milk fat using the formula as given by Gaines 1928) i.e. 4% FCM = (0.4 x g milk yield) + (0.15 x g milk yield x % fat). FCR value during lactation was determined as the amount of DM intake required to produce 1 kg 4% FCM yield.

Milk samples from the consecutive evening and morning milkings were collected from each goat on day seven of each at the first week of lactation. Approximately 30 ml of milk from each goat were composited and stored at +4°C until subsequent analysis for milk composition. Milk compositions of fat, protein, lactose, solids non-fat (SNF), total solids (TS) and specific gravity were analyzed using a Lacto-Scan Milk Analyzer.

Statistical analysis

Data of feed intake, milk yield and milk quality of goats were subjected for analysis of variance using General Linear Model (GLM) procedure of SAS (SAS 2002). If there was a significant difference between treatments, the difference then was compared using Duncan's Multiple Range Test at a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Nutrient intake

Table 2 shows feed (grass, concentrate, forages, and total DM), CP, TDN, NDF, ADF, Ca and P intakes during lactation. The feed (grass, concentrate, mixed forages and total DM) intakes were not significantly different ($P>0.05$) among the treatments. However, there was a significant difference in CP, DIP, Ca and P intakes between treatments ($P<0.05$) but no effect on TDN, NDF, ADF and ratio of roughage to concentrate intakes during lactation period.

Table 2. Average daily nutrient intake of goats fed different levels of protein and energy during lactation

Parameter (g)	Concentrate diets			SEM	P value
	R1 (18%CP, 72%TDN)	R2 (17%CP, 75%TDN)	R3 (16%CP, 78%TDN)		
Grass	420	444	433	37.89	0.340
Concentrate	824	845	768	11.04	0.316
Forage	153	153	153	-	-
Total dry matter	1377	1442	1354	110	0.177
Ratio roughage to concentrate	0.71	0.71	0.79	0.12	0.290
Crude protein	236[a]	237[a]	216[b]	19.56	0.052
Digestible intake protein	174[a]	172[a]	157[b]	14.38	0.040
Total digestible nutrient	982	1043	1011	84.26	0.304
Neutral detergent fiber	713	725	686	46.51	0.211
Acid detergent fiber	452	477	452	27.20	0.096
Calcium	11.75[b]	13.59[a]	12.26[b]	1.25	<0.0001
Phosphorous	8.10[c]	8.26[b]	9.55[a]	0.83	<0.0001

[abc] Values in the same row having different letters show significant ($P<0.05$) difference

Average total daily DM and TDN intakes were not significant (P>0.05) among those three treatments during the first 12 weeks lactation. In this trial, the does were separated with the kids, therefore, nutrient requirement of goats during lactation considered similar to the values recommended by (NRC 2007) for single kid. Furthermore, results of this trial showed that average litter size of goats was 1.4 (data was not shown in the Table). The mean daily total DM and TDN intakes in this trial were less (0.84 times) and similar (0.98 times) to the NRC requirement. According to NRC (2007), daily requirement of DM and TDN for early lactation of a single kid dairy goat at 40 kg of BW and -21 g ADG was 1.67 kg and 1.03 kg, respectively. Kearl (1982) recommended requirement of DM and TDN intakes for the first 10 weeks of lactating goats at 40 kg of BW were 1.90 and 1.05 kg, respectively. From the above results, only the TDN requirement of lactation goats in this trial was closed to Kearl's and NRC's recommendations.

In this trial, different level of protein and energy did not affect DM intake during lactation period. A similar result was reported by Goetsch et al. (2001) that increase energy level had no effect on DM intake of lactating Alpine dairy goats. However, our findings were in contrary from those reported by Rufino et al. (2012), that supplementation of concentrate as sources of protein and energy up to 1.5% BW under grass-pasture increased DM and nutrients intake of goats. Furthermore, Teh et al. (1994) reported that high yielding goats required great amounts of energy during early lactation.

Moreover, different levels of protein and energy in the concentrate diets significantly influenced (P<0.05) the mean daily CP intakes of feed during the lactation period (Table 2). CP intake was higher than Kearl's recommendation Kearl (1982) and NRC requirement (NRC 2007). Requirement of total protein for lactating goats at 40 kg of BW and -20g ADG were 160 g (Kearl 1982) and 89 g UIP 40% and 80 g DIP (NRC 2007) for single kid, respectively. Intakes of Ca and P in this trial were higher than Kearl's recommendation (Kearl 1982) and NRC requirements (NRC 2007). Requirement of Ca and P for lactating goats at 40 kg of BW and -20 g ADG were 5 g and 3.5 g (Kearl 1982), and 5.9 g and 3.9 g for single kid (NRC 2007), respectively.

The main daily intakes of NDF and ADF in this trial were not significant (P>0.05) but the main daily intake of ADF were significantly (P<0.05) different among those concentrate diets. NDF percentages in total DM intakes were 51.78, 50.23, and 50.66% for R1, R2, and R3, respectively. Meanwhile, ADF contents were 32.82, 33.07, and 33.38% for R1, R2, and R3, respectively. NDF and ADF contents of feed intakes were higher than NRC recommendation. The 18 to 20% ADF or 41% NDF was nutritionally adequate for high producing lactating dairy goats (Lu et al. 2008; Mirzaei-Aghsaghali & Maheri-Sis 2011). Moreover, the ratio of roughage to concentrate intakes in this trial was in range as recommended, except for R3 diets which was slightly higher than their recommendations (40 : 60%). Minimum recommended dairy NDF and ADF were 25 to 28% and 19 to 21%, respectively, with at least 75% of this NDF from forages rather than concentrate. Lower dietary fiber level could depress milk fat percentage and increase fat storage in the body of the doe during lactation.

In this trial, different levels of protein and energy in the concentrate diets had no influence (P>0.05) to the ratio of roughage to concentrate intakes during lactation. Intake of concentrates was in the range of 58-61% of total DM intake. These ratios of roughage to concentrate intakes were in the normal range of the feed intakes, except for the R3 diets which was slightly higher than recommended. Those concentrate diets should make up 50-60% of the diets.

From the above results, average TDN intake was adequate to meet the requirement (Kearl 1982; NRC 2007). CP, DIP, NDF, ADF, Ca, and P intakes were higher than the nutrient requirement of lactation goats as recommended by International Feeding System (Kearl 1982; NRC 2007).

Milk yield

Table 3 summarizes effect of different levels of protein and energy in concentrate diets on average daily milk yield at different weeks of lactation, 4% FCM yields, total milk yields for 12 weeks production, FCR, milk constituents and milk composition yields. During milk yield period, different level of protein and energy in concentrate diets, where the three treatments containing 17.14% CP and 71.31% TDN (R1), 16.44% CP and 72.33% TDN (R2), 15.95% CP and 74.67% TDN (R3) of total feeds, did not affect (P>0.05) the average weekly milk yields and the total 12 weeks milk yields.

These results were similar to the result of previous researchers (Bava et al. 2001; Goetsch et al. 2001; Zambom et al. 2012) showing that milk yield was not affected by different level of protein and energy intakes. Bava et al. (2001) reported that milk yield was similar for silage-based control diet and non-forage diet (high CP content) of dairy goats. Goetsch et al. (2001) reported that milk yield in the first 12 weeks of subsequent lactation were not affected by dietary treatment of different level of energy and concentrate or parity of Alpine dairy goats. Zambom et al. (2012) evaluated milk yield of Saanen goats fed diets with soybean hulls replacing ground corn (0, 50, and 100% replacement) in early lactation and the results showed that milk yield was not affected by three different

Table 3. Milk yield, milk composition and milk constituent yields of goats fed different levels of protein and energy

Variables	Concentrate diets			SEM	P value
	R1 (18%CP, 72%TDN)	R2 (17%CP, 75%TDN)	R3 (16%CP, 78%TDN)		
Average daily milk yield					
at 1-4th weeks, ml/d	1720	1560	1649	243	0.483
at 4-8th weeks, ml/d	1441	1294	1611	265	0.130
At 8-12th weeks, ml/d	1166	1010	1245	227	0.189
At 1-12th weeks, ml/d	1442	1288	1502	222	0.225
At 1-12th weeks, g/d	1482	1324	1545	228	0.222
Total milk yield 12 weeks, l	121	108	125	19	0.230
Total milk yield 12 weeks, kg	125	111	130	19	0.222
Avg. Daily 4% FCM yield, g/d	595	531	620	92	0.223
Feed conversion ratio[1]	0.96^{ab}	1.13^{a}	0.94^{b}	0.16	0.068
Milk composition, %					
Fat	5.58	5.82	6.00	0.42	0.312
Protein	2.83	2.82	2.87	0.69	0.556
Lactose	4.78	5.15	4.99	0.38	0.341
Specific gravity	1.028	1.028	1.029	<0.001	0.556
Solid non-fat	8.70	8.86	8.47	0.31	0.180
Total solids[2]	14.28	14.69	14.47	0.52	0.489
Milk constituent yields, g					
Fat	80.71	75.29	90.33	13.13	0.147
Protein	40.71	36.43	43.00	6.26	0.185
Lactose	69.14	66.57	75.17	11.16	0.390
Solid non-fat	126	114	128	19.16	0.413
Total solids[2)]	207	190	218	32.38	0.308

[1]Feed conversion ratio= DMI/4% FCM yield
[2]Total solid= Fat + Solid non-fat
4% FCM (fat corrected milk) = (0.4 x g milk yield) + (0.15 x g milk yield x % fat)
[ab]Values in the same row having different letters differ significantly (P<0.05)

diets containing of 13% CP and 66.49% TDN, 14.5% CP and 63.33% TDN or 15% CP and 57.34% TDN. However, the results of this trial were in contrary with those obtained by other researchers (Sahlu et al. 1995; Park et al. 2010; Souza et al. 2014; Nascimento et al. 2014), who found that the different levels of protein and energy affected the milk yield. Sahlu et al. (1995) reported that milk yield in the subsequently lactation increased quadratically in response to pre-partum CP and TDN concentration. Park et al. (2010) reported that milk yield in the diets of Saanen goats containing 15.19% CP and 62.60% TDN was the highest among the treatments 11.90% CP and 70.08% TDN, 12.73% CP and 67.03% TDN, 16.60% CP and 57.90% TDN. Souza et al. (2012) observed that increasing dietary energy level of Saanen goats using calcium salts of fatty acids changed their lactation curves, resulting in the best milk yield response with 76.18% TDN on DM diets. Nascimento et al. (2014) reported that daily milk yield of dairy goats showed linear improvement with increasing TDN content from 65% to 75% and 85%. The difference in milk yield from other result of previous study might due to the variation of goat responce to the treatment diets, breed, or stages of lactation.

Daily average milk yields of SAPERA in this trial were higher compared to milk yield of Etawah Grade goats (Supriyati et al. 2016) and lower than milk yield of Saanen goats (Gomes et al. 2014; Zambom et al. 2012). Supriyati et al. (2016) reported that average daily milk yields of Etawah Grade goat fed with diets containing 12.6% CP and 70.1% TDN during 12 weeks of lactation was 0.678 kg/d. However, Gomes et al. (2014) reported that average daily milk yields of Saanen goats fed with diets based on soybean meal containing 23% CP during the first 60 days of lactation was 3.29 kg/d. Furthermore, Zambom et al. (2012) reported that average daily milk yields of Saanen goats fed based on soybean hull containing 22% CP and 85% TDN during the 50 days of lactation was 3.64 kg/d. From the above results, it could be concluded that milk yield of SAPERA goats was in the middle range between Etawah Grade and Saanen goats.

Milk constituents and composition yields

Table 3 summarizes milk constituent and composition yield of goats fed different levels of energy and protein. Different levels of energy and protein in concentrate diets had no influences (P>0.05) on milk fat, protein, lactose, specific gravity, SNF, and TS. Milk constituent yields were also not influenced (P>0.05) by the different levels of energy and protein in concentrate diets.

In this trial, milk samples were collected from each goat on day seven of each week of lactation. In this period, milk samples would represent milk quality during whole experiment. As reported by Zeng et al. (1997) that milk sample collection carried out when does were in one to two weeks in lactation. They also reported that daily variation concentration of milk components did not change significantly. Milk components changed depending on the stages of lactation (Zeng et al. 1997) and traits (Silva et al. 2013).

Milk fat and total solids of goats in this trial were in the range reported by Sutama (2009) for Etawah Grade goats under the tropical region, from 4.42 to 6.4%, and 13.62 to 15.72%, respectively. Milk protein and milk lactose of Etawah Grade goats in this trial were less than those reported by Sutama (2009). He also reported that milk protein and milk lactose of Etawah Grade goats were 3.78 to 4.52% and 5.08 to 5.62%, respectively. Protein percentage were less and fat and lactose percentage were higher than those reported by Silva et al. (2013), who worked with Saanen goats; they obtained values of 3.13, 3.78 and 4.25, respectively. Different results from previous studies might due to the differences in feeds, breed and lactation period. From the above results showed that milk content is the most variable nutrient because of the differences between breed, feeding and their interaction.

During milk yield period, different level of protein and energy in concentrate diets, where the three treatments containing 17.14% CP and 71.31% TDN (R1), 16.44% CP and 72.33% TDN (R2), 15.95% CP and 74.67% TDN (R3) of the total feeds did not affect (P>0.05) milk composition and milk constituent yields. However, our findings were in contrary to those obtained by other researchers (Sahlu et al. 1995; Park et al. 2010; Zambom et al. 2012). Sahlu et al. (1995) reported that milk fat percentage increased linearly in response to increased pre-partum energy. Park et al. (2010) reported that the decrease of energy and increase of protein in diets of mid lactation Saanen goats significantly reduced the content of fat milk but the yields of milk protein and lactose increased significantly. Zambom et al. (2012) reported that milk quality of Saanen goats fed diets with soybean hulls in early lactation were not affected by three different diets containing 13%CP and 66.48% TDN, 14.5% CP and 62.33% TDN or 15% CP and 57.34% TDN. Furthermore, Park et al. (2010) suggested that minimum dietary level of protein and energy was 15% CP and 60% TDN in mid lactation for Saanen dairy goats for producing the best milk composition and milk yield constituents.

The different levels of protein and energy response on milk yields and milk composition yields between different research reports might be due to many factors such as forage to concentrate ratio (Tufarelli et al. 2009; Park et al. 2010), breed and traits (Ciappesoni et al. 2004). But forage to concentrate ratio in this trial might not affect milk yield and milk composition since their rations were not significantly different as shown in Table 2. As reported by Tufarelli et al. (2009), ratio 35/65 forage to concentrate provided greater milk yield compared to 50/50 ratio and 65/35 ratios without influencing milk composition during lactation period of Jonica breed goats.

CONCLUSION

Levels of protein and energy in concentrate diets had significant effects on CP, DIP, Ca, P intakes, and FCR but not on DM, TDN, NDF, and ADF intakes during lactation. No significant differences were found in milk yield and milk composition between the different levels of protein and energy in the concentrate diets. This trial suggested that the best feed for lactating SAPERA goats was the mixture of chopped grasses, mixed forages and concentrate diets (16% CP and 78% TDN) with 160 g/kg CP and 750 g/kg TDN of the total DM, produced a milk of 1.55 kg/day with 90 g/day of milk fat, 43 g/day of milk protein and 75 g/day of milk lactose.

REFERENCES

Anwar MM, Ramadan TA, Taha TA. 2015. Serum metabolites, milk yield, and physiological responses during the first week after kidding in Anglo-Nubian, Angora, Baladi, and Damascus goats under subtropical conditions. J Anim Sci. 90:4795-4806.

[AOAC] Association of Official Analytical Chemists. 2012. Official methods of analysis. 19th ed. Maryland (USA): Association of Official Analytical Chemists International.

Bava L, Rapetti L, Crovetto GM, Tamburini A, Sandrucci A, Galassi G, Succi G. 2001. Effect of a non-forage diet on milk yield, energy and nitrogen metabolism in dairy goats throughout lactation. J Dairy Sci. 84:2450-2459.

Ciappesoni G, Prybyl J, Milerski M, Mares V. 2004. Factors affecting goat milk yield and its composition. Czechoslov J Anim Sci. 11:465-473.

[DGLAH] Directorat of General Livestock and Animal Health. 2015. Livestock and animal health of statistics, Jakarta (Indones): Directorat of General Livestock and Animal Health, Ministry of Agriculture.

Gaines WL. 1928. The energy basis of measuring milk yield in dairy cows. Illinois (USA): University of Illinois.

Goetsch AL, Detweiler G, Sahlu T, Puchala R, Dawson L. 2001. Dairy goat performance with different dietary concentrate levels in late lactation. Small Rumin Res. 41:117-125.

Gomes LC, Alcalde CR, de Lima LR, de Lima LS, de Souza R, Possamai APS. 2014. Nutritive value of diets containing inactive dry yeast for lactationg Saanen goats. R Bras Zootech. 43:36-43.

Kearl LC. 1982. Nutrient requirement of ruminants in developing countries. Logan Utah (USA): International Institute of Feedstuffs, Utah Agricultural Experiment Station, Utah University.

Krishnamoorthy U, Moran J. 2011. Rearing young ruminants on milk replacers and starter feeds. In: Makkar HPS, editor. Manual of animal production and health. 13th ed. Rome (Italy): Food Association Organization.

Lu CD, Kawas JR, Mahgoub OG. 2008. Review recent advancements in fiber digestion and utilization in goats. Trop Subtrop Agroecosys. 9:65-72.

Martínez-Marín AL, Pérez-Hernández M, Pérez-Alba LM, Carrión-Pardo D, Gómez-Castro AG. 2011. Prediction of energy requirements of Murciano-Granadina pre-ruminant female kids using the National Research Council. S Afr J Anim Sci. 41:345-349.

Mellado M, Meza-Herrera CAF, Arévalo JR, De Santiago-Miramontes MA, Rodríguez A, Luna-Orozco JR, Veliz-Deras FG. 2011. Relationship between litter birth weight and litter size in five goat genotypes. Anim Prod Sci. 51:144-149.

Mirzaei-Aghsaghali A, Maheri-Sis N. 2011. Importance of "physically effective fiber" in ruminant: A review. Ann Biol Res. 2:262-270.

Nascimento TVC, Miranda MS, Barros CHSC, Souza TTS, Lopes-Júnior ES, Voltolini TV, Moraes SA, Nogueira DM, Cordeiro MF. 2014. Return of postpartum ovarian activity in dairy goats supplemented with different levels of energy. Rev Bras Saúde Prod Anim. 15:1061-1071.

[NRC] National Research Council. 1981. Nutrient requirement of goats. Washington DC (USA): National Academy Press.

[NRC] National Research Council. 2007. Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids. 7th ed. Washington DC (USA): National Academy Press.

Park JK, Kim JD, Kim JA, Yoo JS, Bae GS, Kim CH. 2010. Effects of different dietary energy and protein levels on milk yield in Saanen dairy goats in mid lactation. J Korean Grassl Sci. 30:35-42.

Praharani L. 2014. Milk yield of Anglo-Nubian, Saanen x Etawah Grade and Etawah Grade raised in the same environment. In: Subandriyo, Kusmartono, Santosa KA, Kurnianto E, Purnomoadi A, Sodiq A, Wiryawan KG, Darodjah S, Inounu I, Darmono, Priyanti A, Wynn P, Han JL, Tay-Hsu J, Idrus Z, editors. Proceedings the 16th Asian-Australasian Associations of Animal Production Societies. [Yogyakarta (Indonesia)]: Universitas Gadjah Mada. p. 1527-1530.

Rufino MOA, Alves AA, Rodrigues MM, Moura RL, Cavalcante ACR, Rogerio MCP. 2012. Goat milk yield and quality on Tanzania-grass pastures, with supplementation. Acta Sci Anim Sci. 34:417-423.

Sahlu T, Hart SP, Le-Trong T, Jia Z, Dawson L, Gipson T, Teh TH. 1995. Influence of pre-partum protein and energy concentrations for dairy goats during pregnancy and early lactation. J Dairy Sci. 78:378-387.

[SAS] Statistics Analysis System. 2002. SAS/STAT user's guide. Version 9.1. North Carolina (USA): SAS Institute Inc., Cary.

Silva FG, Brito LF, Torres RA, Riberio Junior JI, Oliviera HR, Caetano GC, Rodrigues MT. 2013. Factors that influence the test day milk yild and composition. Genet Mol Res. 12:1522-1532.

Souza de R, Alcalde CR, Oliveira CAL, Molina BSL, Macedo FAF, Gomes LC, Hygino B, Possamai APS. 2014. Lactation curves and economic results of Saanen goats fed increasing dietary energy levels obtained by addition of calcium saltas of fatty acids. R Bras Zootech. 43:73-79.

Supriyati, Praharani L, Budiarsana I-GM, Sutama I-K. 2014a. Pengaruh perbedaan level protein dan energy pada konsentrat terhadap kinerja kambing betina muda

Peranakan Etawah. In: Pamungkas D, Widiawaty Y, Noor SM, Purwantari ND, Widiastuti R, Brahmantiyo B, Herawati T, Kusumaningsih A, Handiwirawan E, Puastuti W, editors. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. [Bogor (Indones)]: Indonesian Center for Animal Research and Development. Hlm. 449-456.

Supriyati, Praharani L, Budiarsana IGM, Sutama I-K. 2014b. Effect of different protein and energy levels in concentrate on Anglo Nubian goat performance. In: Subandriyo, Kusmartono, Santosa KA, Kurnianto E, Purnomoadi A, Sodiq A, Wiryawan KG, Darodjah S, Inounu I, Darmono, Priyanti A, Wynn P, Han JL, Tay-Hsu J, Idrus Z, editors. Proceeding of the 16th AAAP Animal Science Congress. [Yogyakarta (Indonesia)]: Universitas Gadjah Mada. p. 1890-1894.

Supriyati, Budiarsana I-GM, Praharani L, Krisnan R, Sutama I-K. 2016. Effect of choline chloride supplementation on milk yield and milk composition of Etawah grade goats. J Anim Sci Technol. 58:1-12.

Sutama I-K. 2009. Productive and reproductive performances of female Etawah Crossbred goats in Indonesia. Indones Bull Anim Vet Sci. 19:1-6.

Teh TH, Trung LT, Jia ZH, Gipson TA, Ogden KB, Sweeney TF. 1994. Varying amounts of rumen-inert fat for high producing goats in early lactation. J Dairy Sci. 77:253-258.

Tufarelli V, Dario M, Laudadio V. 2009. Forage to concentrate ration in Jonica breed goats: Influence of lactation curve and milk composition. J Dairy Sci. 76:124-128.

Zambom MA, Alcalde CR, Kazama DCS, Martins EN, Hashimoto JH, Matsushita M, Ramos CECO, Grande PA. 2012. Soybean hulls replacing ground corn in diets for early lactation Saanen goats: Intake, digestibility, milk yield and quality. R Bras Zootec. 41:1-8.

Zeng SS, Escobar EN, Popham T. 1997. Daily variations in somatic cell count, composition, and production of Alpine goat milk. Small Rumin Res. 26:253-260.

Histological Changes of Liver Tissue and Serobiochemical Relation in Does with Pregnancy Ketosis

Azmi AFM[1], Ghani AAA[1], Saadan AS[1], Mokrish A[1], Lai KS[2], Zamri-Saad M[1], Zuki AB[1], Hassim HA[1]

[1]*Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia*
[2]*Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia*
E-mail: haslizaabu@upm.edu.my

(received 04-04-2016; revised 10-06-2016; accepted 20-06-2016)

ABSTRAK

Azmi AFM, Ghani AAA, Saadan AS, Mokrish A, Lai KS, Zamri-Saad M, Zuki AB, Hassim HA. 2016. Perubahan histologi jaringan hati dan hubungan serum biokimia pada kambing yang mengalami kebuntingan ketosis. JITV 21(2): 96-100. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1357

Perubahan histologi hati kambing yang mengalami kebuntingan ketosis dikarakterisasi pada penelitian ini. Dua puluh kambing bunting pada hari ke-80 digunakan pada percobaan ini. Sepuluh kambing bunting diberi pakan rumput (Napier) dan konsentrat bersama air secara *ad libitum* dikategorikan sebagai kambing bunting yang sehat dan 10 kambing lainnya yang menunjukkan gejala-gejala klinik dan subklinik ketosis dikategorikan sebagai kambing bunting tidak sehat. Biopsi hati dilakukan saat gejala klinik muncul. Beta-Hydroxybutyrate (BHBA), asam lemak bebas, dan gula ditentukan dosisnya. Persiapan histologi menunjukkan persamaan insidensi dan intensitas steatosis hati ringan bersama vakuolasi seluler yang lebih rendah di dalam hepatosit kambing bunting yang sehat. Hampir semua kambing bunting yang menderita ketosis (n=8/10) mempunyai jumlah kadar tetesan lipid yang besar di dalam setiap hepatosit di semua asinus hati bersama kadar selular vakuolasi yang tinggi, serta berhubungan dengan peningkatan kadar BHBA dan asam lemak bebas saat terjadi penurunan kadar gula dalam darah.

Kata Kunci: Asam Lemak Bebas, Beta-Hydroxybutyrate, Kambing, Biopsi Hati, Kebuntingan Ketosis

ABSTRACT

Azmi AFM, Ghani AAA, Saadan AS, Mokrish A, Lai KS, Zamri-Saad M, Zuki AB, Hassim HA. 2016. Histological changes of liver tissue and serobiochemical relation in does with pregnancy ketosis. JITV 21(2): 96-100. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1357

Histological changes of liver in does with pregnancy ketosis were characterized. Twenty pregnant does at day 80 of pregnancy were used for this experiment. A total of 10 does were fed by grass (Napier) and goat concentrate with water ad libitum. Those 10 goats considered as healthy pregnant goat, and another 10 goats showing clinical and subclinical signs of ketosis considered as unhealthy pregnant does. Liver biopsies were performed when clinical signs appeared. Beta-Hydroxybutyrate (BHBA), free fatty acid (FFA), and glucose were dosed. Histological preparation revealed similar incidence and intensity of mild liver steatosis with lower cellular vacuolation in hepatocyte presence in healthy late pregnant does. Almost all of the pregnant does with ketosis state (n=8/10) had large amount of small lipid droplets in almost every hepatocyte over the whole liver acinus with higher number of cellular vacuolation, and related with higher BHBA and FFA levels while low in glucose level.

Key Words: Beta-Hydroxybutyrate, Does, Free Fatty Acid, Liver Biopsies, Pregnancy Ketosis

INTRODUCTION

Recently, there was a sharp increase in goat milk and meat demand in Malaysia, particularly in the last three decades due to rapid economic and population growth, with the resultant effects of urbanization, income growth and change of consumer preference (Bisant 2010). Nevertheless, scientifically based on goat farms and industry information in Malaysia, supply is extremely limited to meet the sudden surge of goat milk and meat demand. Urgent issues faced by goat farmers were the improper rearing management, feed and feeding, diseases and marketing (Jamaludin et al. 2012). Pregnancy ketosis has been recognized as one of the common metabolic diseases influencing goat meat and milk production (Bani-Ismail et al. 2009). Pregnancy ketosis commonly occured in goat or sheep during the late stage of gestation with a low morbidity rate (2-5%) but a high mortality rate (80%) (Brounts et al. 2004; Zamir et al. 2009; Brozos et al. 2011). The main cause of pregnancy ketosis in goats was a disturbance of carbohydrate metabolism due to high demand for glucose by the developing fetuses in the last trismester of pregnancy, resulting negative energy balance

(Schlumbohm & Harmeyer 2004). As a fetal requirement of glucose exceeding dietary energy intake, increase in lipolysis led to an augmented synthesis of ketone bodies (BHBA) to maintain metabolic homeostasis (Pethick & Lindsay 1982; Sargison et al. 1994). The disturbance of carbohydrate metabolism generates high plasma free fatty acids and ketone bodies level. It was suggested that fatty liver due to lipolysis interferes with hepatic gluconeogenic capacity, thus ketosis and fatty liver disease would play a central role in pregnancy ketosis (Herdt 2000).

Histopathological reports related to the disease are usually referred to data obtained from liver biopsy or during post mortem examination. Many workers claimed that during ketosis, this condition occured to some tissues of animal i.e. cerebral and cerebellar neuronal necrosis and vacuolation, early structural maturity of placenta, and liver steatosis (Snook 1939; Mitchell & Stratford 1987; Marteniuk & Herdt 1988; Jeffrey & Higgins 1992; Andrews 1997). Although being a rather commonly studied disease, there are no previous researchs which access evolution of histological changes of liver tissue during pregnancy ketosis in relation to serobiochemical changes.

The aim of this work was to assess and characterize evolution of histological changes of liver in does induced to pregnancy ketosis by puncture biopsy method. It also studied relation of these changes with glucose, BHBA and free fatty acid levels to consider then as possible prognostic indicator of hepatic damage in this disease and determination severity of pregnancy ketosis in does.

MATERIALS AND METHODS

Animal selection

Twenty crossed Boer goats aged between 1 to 3 years old are studied from December 2014 to December 2015. A total of 10 clinically healthy does were used as the control group while another 10 does with clinical signs of ketosis considered as unhealthy animal. All the stages of pregnancy were confirmed by ulrasonography at day 80. All does carried singleton pregnancy. Sampling procedure was approved by Ethical Committee for Animal Experiments, Universiti Putra Malaysia.

History and selection of cases

Examined goats were raised in small-scale flocks in rural areas. Those pregnant goats were fed on natural grasses (Napier), concentrate with supplementation of roughage and running water *ad libitum*. They were diagnosed with suspected pregnancy ketosis via clinical histories and through physical examination.

Biochemical examination

Blood samples were collected from all does under experimentation; the first sample collected in plain Vacutainer tubes and used for serobiochemical examination (eg: glucose) by sending it to clinical pathology lab, UPM. Next, sample was collected in EDTA tubes and plasma was separated using centrifuge under 3000x rpm. All those plasma were stored at -20°C until further utilization. Beta-hydroxubutyrate (BHBA) and free fatty acids (FFA) were examined using plasma sample. BHBA and FFA were examined using spectrophotometric following standard methods using commercial ELISA kits (CAYMAN, USA).

Histopathological examination

Samples were collected from healthy goats as control and goats suffering severe ketosis. Liver samples were collected using liver biopsy and local anesthesia was given before the sample collection. All liver specimens obtained by biopsy were processed and stained using hematoxylin and eosin. The present of fat droplet in liver cell and severity of cellular vacuolar degeneration was evaluated at 20 times and 40 times of power magnification and scored according to Stockhaus et al. (2004): 1 = small amounts in a minority of cells, 2 = variable amounts in a majority of cells, and 3 = large amounts in almost every hepatocyte.

Statistical analysis

All the analysis were done with SPSS version 20.0 software and data represent as a mean were considered significantly different when $P<0.05$.

RESULTS AND DISCUSSION

Liver biopsy is the most practical method for fatty liver diagnosis. Transthoracic needle biopsies used for safely taken and easily through the tenth interescostal space at level of the greater trochanter. Samples of biopsy taken should be evaluated qualitatively for histological means using grading system. Figure 1 shows the healthy liver of pregnant does with absence of fat droplet and presence of steatotic changes with mild condition. This study was inline with Rook (2000) who reported that healthy pregnant showed a largely unchanged during the experiment and mild steatotic changes recorded in those does seemed to be a normal feature during late pregnancy. Figure 2 shows the goats liver under subclinical ketosis which fatty acid accumulation was presence in every single of liver cell. At this stage, the majority of affected does was poorly extended degeneration.

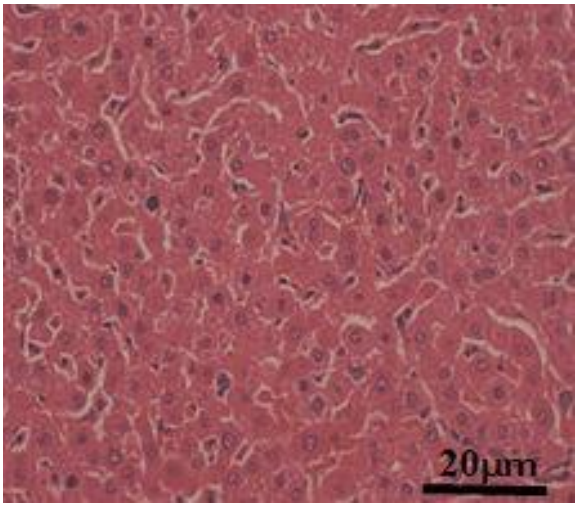


Figure 1. Healthy goat liver condition. Photomicrograph of healthy liver with absence of lipid droplets in almost every hepatocyte. Healthy goats liver under 40x magnification. Scale bar = 20 µm.

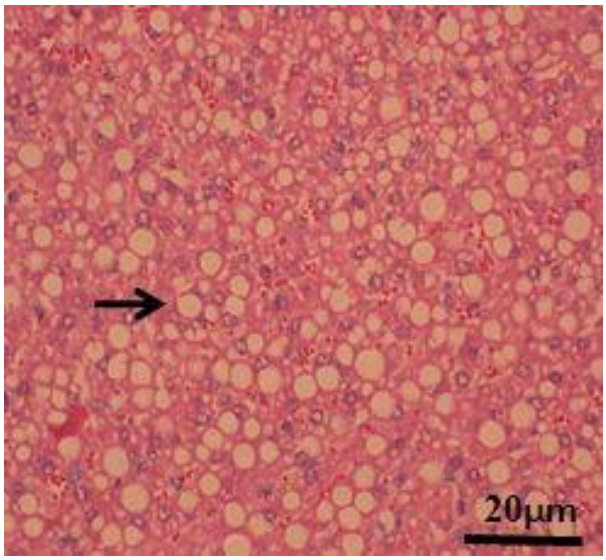


Figure 2. Goats liver under subclinical ketosis. Severe liver contains large amount of lipid droplets in almost every hepatocyte. Fatty liver pregnancy ketosis goats under 40x magnification. Scale bar = 20 µm. Arrows show the presence droplet of fat inside hepatocyte cell. Photomicrograph of liver of goat with diffuse hepatic lipidosis showing macrovacuales vacuoles pushing the nucleus to one side which signet ring appearance.

This description and corresponding illustration given by Jaeschke et al. (2002) fit with this study stated microvesicular degeneration caused by acute starvation due to fatty acid accumulation. During ketosis, hepatic degeneration during late pregnancy in ketosis doe was a microvesicular type. Microvesicular types of steatosis has been described and corresponding illustration given by Snook (1939) and were obtained from a slaughtered ewe that comatose due to pregnancy ketosis.

Histological investigation also demonstrated liver degeneration incidence and severity in both groups. Healthy goats in this experiment remained largely and unchanged on cellular vacuolation with score 1.22±0.44 (which score around 1 and 2) and mild steatotic changes observed. According to Rook (2000), mild steatotic changes observed during the late pregnancy considered as normal. However, a ketosis goat in this experiment showed higher liver degeneration and cellular vacuolation which grade of cellular vacuolation around 2.89±0.33 (which score around 2 and 3). Higher cellular vacuolation during pregnancy ketosis also supported by Cal et al. (2009) which enhanced lipidic mobilization in starved animals would have caused a higher incidence of vacuolation and was significantly enhanced at the end of the starving period. All samples with degeneration diagnosis of both groups corresponded to the cellular vacuolation (Table 1), and alterations such as steatohepatitis or necrosis were not detected.

Table 1. Number of goats with and without liver degeneration corresponding degree of cellular vacuolation

Groups	Number of animal (n)	Cellular vacuolation
Healthy goats	10	1.22±0.44
Subclinical pregnancy ketosis goats	10	2.89±0.33

*P<0.05

In this study, serum biochemical profiles in does were characterized as subclinical pregnancy ketosis. Depression and teeth grindings were the first signs observed in both mild and advance pregnancy ketosis goats. This study indicated that the goat with subclinical pregnancy ketosis, as determined by ketonemia, were more likely to have hypoglycaemia and azotemia compared to non ketotic pregnant goats during the third trimester pregnancy. Laboratory findings showed the presence of high number of BHBA and FFA of clinically affected goats in the plasma which 1.37±0.47 mmol/L and 0.98±0.22 mmol/L, respectively (Table 2). The increase of FFA concentration in plasma of affected goats could be attributed to the increase of mobilization of fatty acids from adipose tissue in response to a requirement for endogenous substrate for energy production during pregnancy (Noble et al. 1971). Indeed, elevation level of FFA due to ketosis inside plasma reflected the formation of fatty liver in pregnant does. It also suggested that plasma FFA would be the most useful index of degree of under

nourishment in pregnant does (Russel et al. 1976). Thus, increase of FFA levels during ketosis state reflected the elevation of BHBA level which was 1.37±0.47 mmol/L. Previous study reported that goat having range BHBA around 0.8 to 1.6 mmol/L (Sadjadian et al. 2013), >0.86 mmol/L (Bani-Ismail et al. 2009), >1.1 mmol/L (Brozos et al. 2011) were considered as a subclinical pregnancy ketosis. Sadjadian et al. (2013) also reported that an excessive negative energy balance prepartum could be identified by presence of hyperketonemia. Elevation of BHBA level also was able to inhibit hepatic gluconeogenesis, and thus further increase maternal hypocglycaemina (Schlumbohm & Harmeyer 2004). The result also demonstrated that glucose level of ketotic goats 1.13±0.48 mmol/L was lower than healthy goat 2.88±0.33 mmol/L (Table 2). Glucose is the main sources for supplying energy in the body. Glucose level shows relation to the animal energy status which its values was negatively related with a negative energy balance. These results showed an increase in mobilization of glucose as well as free fatty acids to be used in thermogenic process (Terashima et al. 1982). In addition, data collected could be used by veterinarians to aid in clinical investigation of herds and individual goats in late pregnancy, and help in the understanding of histologic and pathophysiologic changes occured in this species.

Table 2. Serum metabolic constituents in does in late pregnancy, with and without (healthy) subclinical pregnancy ketosis

Constituents	Subclinical ketosis (n =10)	Healthy goats (n=10)
Glucose (mmol/L)	1.13±0.48	2.88±0.33
Beta Hydroxybutyrate (mmol/L)	1.37±0.47	0.13±0.06
Free Fatty Acids (mmol/L)	0.98±0.22	0.18±0.08

*P<0.05

CONCLUSION

It was concluded that a pregnancy ketosis during late pregnancy in does could produce fatty liver with higher cellular vacuolation. Severity of liver damage and presence of fat droplet was found to be associated with higher number of FFA and BHBA and lower values of glucose levels in the blood plasma.

ACKNOWLEDGEMENTS

Authors would like to thank farmers in all Ladang Angkat Universiti Putra Malaysia (UPM) for allowing us to examine their herds and collecting samples. We would also like to thank staff of Universiti Veterinary Hospital (UVH) for facilitating laboratory evaluation of the plasma samples in their laboratories. This study also was financially supported by the FRGS Grant and Ministry of Higher Education, Malaysia.

REFERENCES

Andrews A. 1997. Pregnancy toxaemia in the ewe. In Pract. 19:306-314.

Bani-Ismail ZA, Al-Majali AM, Amireh F, Al-Rawashdeh OF. 2009. Metabolic profiles in goat do in late pregnancy with and without subclinical pregnancy toxemia. Vet Clin Pathol. 37:434-437.

Bisant K. 2010. Consumer preference for goat meat in Malaysia: Market opportunities and potential. J Agribus Mark. 3:40-55.

Brounts S, Hawkins J, Baird AN, Glickman L. 2004. Outcome and subsequent fertility of sheep and goats undergoing caesarean section because of dystocia: 110 cases (1981–2001). J Am Vet Med Assoc. 224:275-279.

Brozos C, Mavrogianni VS, Fthenakis GC. 2011. Treatment and control of peri-parturient metabolic diseases: Pregnancy toxemia, hypocalcemia, hypomagnesemia. Vet Clin North Am Food Anim Pr. 27:105-113.

Cal L, Borteiro C, Benech A, Rodas E, Abreu MN, Cruz JR, Montana G. 2009. Histological changes of the liver and metabolic correlates in ewes with pregnancy toxemia. Arq Bras Med Vet Zootech. 61:306-312.

Herdt TH. 2000. Ruminant adaptation to negative energy balance. Vet Clin North Am Food Anim Pr. 16:215-230.

Jaeschke H, Gores GJ, Cederbaum AI. 2002. Mechanisms of hepatotoxicity. Toxicol Sci. 65:166-176.

Jamaludin AA, Idris K, Roslaini R. 2012. Challenges facing dairy goat farmers in Malaysia. In: Abdullah R, Omar MA, Makkar H, Otte J, Rajion MA, Alimon AR, Boo LJ, Kam HA, Li CW, editors. Proceeding First Asia Dairy Goat Conference. Kuala Lumpur (Malays): Universiti Putra Malaysia and The Food and Agricultural Organization of the United Nations. p. 11-13.

Jeffrey M, Higgins RJ. 1992. Brain lesions of naturally occurring pregnancy toxaemia of sheep. Vet Pathol. 29:301-307.

Marteniuk JV, Herdt TH. 1988. Pregnancy toxemia and Ketosis of ewes and does. Vet Clin North Am Food Anim Pr. 4:307-315.

Mitchell GM, Stratford BF. 1987. The morphology of the ovine placenta in pregnancy toxaemia. Aust Vet J. 64:221-223.

Noble RC, Steel W, Moore JH. 1971. The plasma lipids of the ewe during pregnancy and lactation. Res Vet Sci. 12:47-53.

Pethick DW, Lindsay DB. 1982. Metabolism of ketone bodies in pregnant sheep. Br J Nutr. 48:549-563.

Rook JS. 2000. Pregnancy toxemia of ewes, does, and beef cows. Vet Clin North Am Food Anim Pr. 16:293-317.

Russel AJF, Donery JN, Reid RL. 1976. The use of biochemical parameters in controlling nutritional state in pregnant ewes and the effect of undernourishment during pregnancy on lamb birth-weight. J Agric Sci Camb. 68:359.

Sadjadian R, Seifi HA, Mohri M, Naseian AA, Farzaneh N. 2013. Variations of energy biochemical metabolites in periparturient dairy Saanen goats. Comp Clin Pathol. 22:449-456.

Sargison ND, Scott PR, Penny CD, Pirie RS, Kelly JM. 1994. Plasma enzmes and metabolites as potential prognostic indices of ovine pregnancy toxaemia a preliminary study. Br Vet J. 150:271.

Schlumbohm C, Harmeyer J. 2004. Hyperketonemia impairs glucose metabolism in pregnant ewes. J Dairy Sci. 87:350-358.

Snook LC. 1939. Fatty infiltration of the liver in pregnant ewes. J Physiol. 97:238-249.

Stockhaus C, Van Den Ingh T, Rothuizen J. 2004. A multistep approach in the cytologic evaluation of liver biopsy samples of dogs with hepatic diseases. Vet Pathol. 41:461-470.

Terashima Y, Tucker RE, Deets LE, Degregorio RM, Muntifering RB, Mitchell CE. 1982. Plasma magnesium levels as influenced by cold exposure in fed or fasted sheep. J Nutr. 112:1914-1920.

Zamir S, Rozov A, Gootwine E. 2009. Treatment of pregnancy toxaemia in sheep with flunixin meglumine. Vet Rec. 165:265-266.

Methane Emission Factors for Enteric Fermentation in Beef Cattle using IPCC Tier-2 Method in Indonesia

Widiawati Y[1], Rofiq MN[2], Tiesnamurti B[3]

[1]*Indonesian Research Institue of Animal Production, PO Box 221, Bogor 16002*
[2]*Center for Agricultural Production Technology-Agency for the Assessment and Application Technology Building 610, Puspiptek Region-Serpong 15314*
[3]*Indonesian Center for Animal Research and Development*
E-mail: rayeni@pertanian.go.id

(received 04-04-2016; revised 13-06-2016; accepted 24-06-2016)

ABSTRAK

Widiawati Y, Rofiq MN, Tiesnamurti B. 2016. Pengaruh emisi gas metana untuk fermentasi enterik pada sapi potong menggunakan metode Tier-2 IPCC di Indonesia. JITV 21(2): 101-111. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1358

Gas metana dari enterik fermentasi termasuk ke dalam sub-kategori yang menjadi perhatian di sektor pertanian oleh UNFCCC. Oleh karena itu, Indonesia perlu mengembangkan faktor emisi (FE) untuk gas metana enterik dengan menggunakan metode Tier-2 sebagai FE spesifik negara**.** Populasi sapi potong merupakan populasi terbesar diantara ternak ruminansia besar lainnya. Sehingga memberikan kontribusi gas metana enterik yang cukup signifikan diantara ternak lainnya. Tujuan dari kegiatan adalah untuk menentukan nilai FE metana enterik dari sapi potong dengan menggunakan metode Tier-2. Nilai FE ini selanjutnya digunakan untuk menghitung emisi metana enterik dari sapi potong di Indonesia**.** Data populasi sapi diambil dari BPS, data kandungan energi pakan, konsumi dan kecernaan pakan dikompilasi dari hasil analisa laboratorium dan data yang sudah dipublikasi. Sapi lokal mempunyai nilai FE yang berbeda diantara sub-kategori lainnya dan berkisar dari 18,18 sampai 55,89 Kg CH_4/ekor/tahun, dengan rata-rata 36,75 Kg/ekor/tahun. Nilai FE untuk sapi impor (25,49 kg CH_4/ekor/tahun) lebih rendah daripada rata-rata sapi lokal. Secara keseluruhan, nilai FE nasional untuk sapi potong yang dihitung dengan metode Tier-2 adalah 33,14 kg CH_4/ekor/tahun lebih rendah dari default FE yang terdapat dalam buku IPCC 2006 untuk wilayah Asia (47 kg CH_4/ekor/tahun). Kesimpulannya adalah bahwa nilai FE yang dihitung menggunakan metoda Tier-2 dengan menggunakan data lokal Indonesia lebih mendekati kondisi peternakan yang sebenarnya di Indonesia. Penelitian kedepan masih diperlukan terkait penentuan nilai FE untuk setiap bangsa sapi potong dan setiap system pemeliharaan yang sangat bervariasi diantara provinsi di Indonesia.

Kata Kunci: Faktor Emisi Metana, Fermentasi Enterik, Sapi Potong, Tier-2 IPCC

ABSTRACT

Widiawati Y, Rofiq MN, Tiesnamurti B. 2016. Methane emission factors for enteric fermentation in beef cattle using IPCC Tier-2 method in Indonesia. JITV 21(2): 101-111. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1358

Methane emission from enteric is a sub-category considered under the Agriculture sector greenhouse gas emissions by UNFCCC. Thus Indonesia developed calculation on enteric CH_4 EF for ruminant using Tier-2 method as country-specific emission factors (EF). Indonesia has huge amount of beef cattle population, which contributes significant amount to national enteric methane emission. The aim of this study was to estimate enteric methane EF for beef cattle in Indonesia using IPCC Tier-2 method. The EF generated from this study is then used to estimate the methane emitted from beef cattle. Data on beef cattle population was obtained from CSA, data on energy content of feed, feed intake and digestibility were compiled from laboratory analysis and published paper. Equations were adopted and followed the instruction of IPCC 2006. Local cattle has different CH_4 EF among each sub-category, which are ranging from 18.18 to 55.89 Kg head-1 yr-1, with the average of 36.75 head-1 yr-1. Imported beef cattle has lower CH_4 EF (25.49 kg head-1 yr-1) than the average for local beef cattle. Overall, the national CH_4 EF of beef cattle calculated by using IPCC Tier-2 method in Indonesia is 33.14 head-1 yr-1. The value is lower than default EF from IPCC for Asia country (47 kg head-1 yr-1). The conclussion is enteric CH_4 EF for beef cattle in Indonesia calculated using Tier-2 method shows the real livestock system in Indonesia condition. Further researchs needed to be addressed are calculation of EFs for various breeds and feeding systems, since large variations of breeds and types of feed among provinces in Indonesia.

Key Words: Methane Emission Factors, Enteric Fermentation, Beef Cattle, IPCC Tier-2

INTRODUCTION

Methane (CH_4) is one of greenhouse gasses (GHG) produced from anthropogenic activities, including from livestock activity. Other GHG are dinitrooxide (N_2O), carbon dioxide (CO_2) and chlorofluorocarbon (CFC) (Gerber et al. 2013). Among other gasses, CH_4 is the second to CO_2 in term of its contribution to climate change (Lassey 2007). In year 2012, the total GHG emission from Indonesia becomes 1,453,957 Gg CO_2-e or increased by 45% from the GHG emission in year 2000. Agricultural sector is contribute for only 7.8%, after contribution of energy (34.9%) for national GHG. Contribution of GHG from livestock to agriculture sector is accounted for about 27,465 Gg CO_2-e or 24.4 %, including CH_4, CO_2 and N_2O from enteric fermentation and manure management by Ministry of Environment. Among the livestocks, ruminant contributes for about 94% and non ruminant contributes for about 6% of CH_4 emitted to the atmosfer. In ruminant group, beef cattle is the larger contributor (69.41%) due to the large population and body size.

Methane is a trace gasses that formed during rumen fermentation process of feed (Rong-Zhen et al. 2016). Conversion of feed materials into CH_4 in the rumen involves the integration of several species of microorganisms ended metanogenesis bacterial activity. The main microorganisms in the rumen perform hydrolysis of proteins, carbohydrates, and polymer plant cell walls into acidic amino acids and simple sugars. The products are fermented into Volatile Fatty Acid (VFA), and hydrogen (H_2) as the secondary products of rumen microorganisms in the digestive system. The main components of VFA are acetic, propionic and butyric acid. These acids are absorbed and used by ruminants as sources of energy for their productivity. Other secondary products (H_2) are then converted to CH_4 in the present of CO_2. The amount of CH_4 formed during rumen fermentation depend on feed quality (energy dan fibre content) and the level of intake. Increasing in dietary intake followed by nutrient intake will affect rumen environment as well as digestibility of the diet, and therefore CH_4 produced (Hammond et al. 2013; Gregorini et al. 2010; Dijkstra et al. 2011).

Due to its contribution to the global warming, it is necessary to mitigate CH_4 production during rumen fermentation of feed. The effectiveness of selection on the mitigation technique to reduce CH_4 emission from enteric fermentation significantly depend on the accuracy of country-specific national GHG inventories that can be used as bench mark for the initial value of CH_4 emission. However, the previous review of national GHG inventory in Indonesia was calculated by using Tier-1 method of IPCC. In this method, GHG emission was calculated by multiplied livestock population with emission factor (EF). The EF values used in the calculation were the default EF suggested by IPCC for Asia countries. The GHG emission estimated by using Tier-1 method shows high uncertainties largely caused by gaps in activity data and the use of IPCC default EFs that did not reflect a real condition of Indonesia's livestock (Widiawati 2013).

Estimation of CH_4 emission from livestock has been directed by a guideline published by The Intergovernmental Panel for Climate Change (IPCC) since 1996 and renewed in year 2006 (IPCC 2006). There are three Tiers methods are provided in IPCC guidelines book, which can be used to estimate methane emitted from livestock. They are Tier-1, Tier-2 and Tier-3 methods. Tier-1 is the basic method, Tier-2 is an intermediate method and Tier-3 is the most demanding in terms of complexity and data requirements. Tiers-2 and Tier-3 methods are referred to higher Tier method and generally considered to be more accurate. The three methods require some factors, such as value of EF, livestock sub-categories based on their level productions, and annual livestocks population. When higher levels of methods (Tier-2 and Tier-3) are used, data on feed intake and feed quality are required. These methods suggested by IPCC, represent as Tier method, have a level of methodological complexity. Selection of Tier method used to estimate CH_4 emission is depend on some factors. When the enteric fermentation from ruminant in the country becomes a key-catagory, the Tier-2 method must be used. According to the Key-Category Analysis (KCA) from IPCC (2006), enteric fermentation is one of categories that take priority in GHG inventory because of the large emission/absorption has a major influence on the total of GHG inventory. Moreover, beef cattle in Indonesia is a species that contribute the highest amount of CH_4 among other ruminant species (Widiawati 2013), thus it becomes a key-category. Therefore estimation of methane emitted from beef cattle must use Tier-2 or Tier-3 methods.

In Indonesia, available data in beef cattle is enough to decide the using of Tier-2 method, where the data on sub-category of beef cattle, feeding situation information, feed intake and feed quality for beef cattle could become a Category Key in enteric CH_4 emissions when using Tier-2 method. Other requirements in Tier-2 method are data enrichment characteristics of livestock, feed intake and energy consumption. When Tier-2 method is used to estimates methane emitted from livestock, the EF value used in the method must use a country specific EF value. Therefore the main objective of this study is to develop country specific EF for CH_4 from enteric fermentation of beef cattle. This EF is then used to estimate the methane emitted from beef cattle in Indonesia by using Tier-2 method. Estimation of national methane emitted from beef cattle

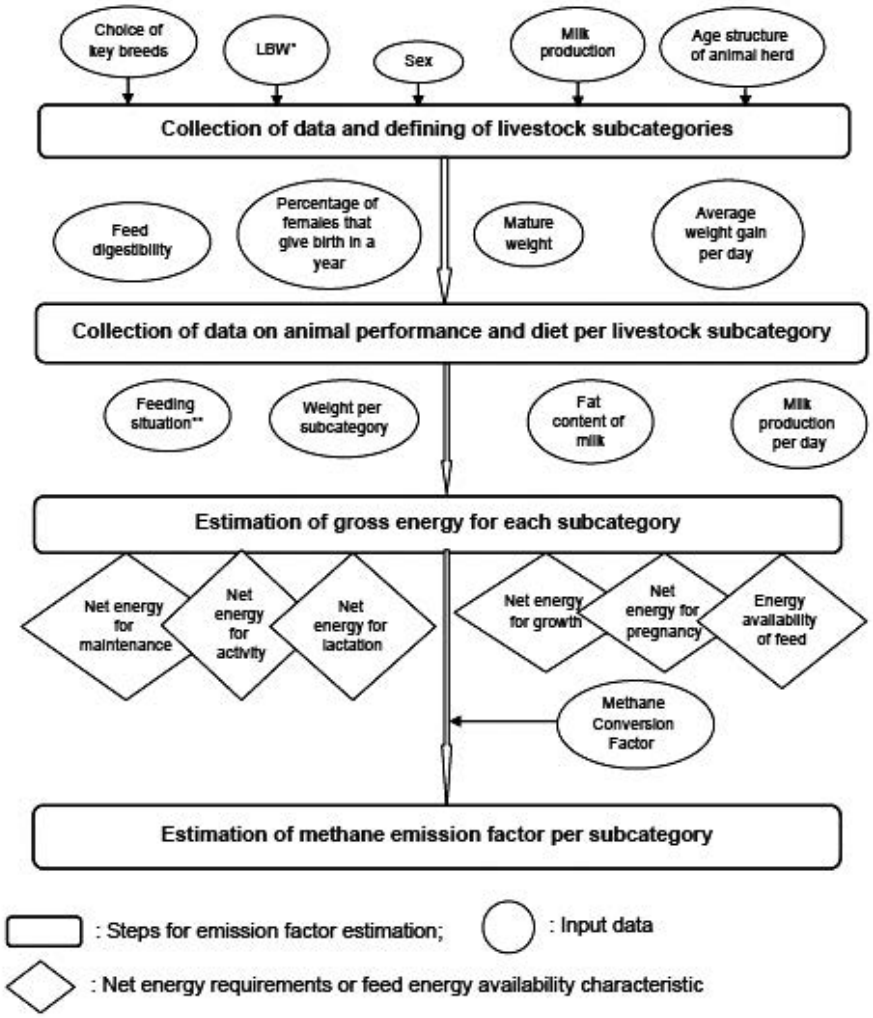
is required to identify contribution of livestock to nasional GHG emission.

MATERIALS AND METHODS

To develope country specifis EF values required in Tier-2 method, some factors are required. They are livestock population based on the level production (sub-categories), live weight, feed intake, and energy content of the feed. Beef cattle population in Indonesia is divided into five sub-categories based on the level production of the animals. Data collection and analysis to develope EF values were undertaken by follows some steps as outlined in IPCC Tier-2 method (Figure 1).

Characterization of beef cattle population and performance data subcategories

Data on beef cattle population was collected from Statistics Indonesia (CSA 2015) as the Central agency on statistic in Indonesia. Beef cattle sub-categories based on production levels were collected from Directorate General of Livestock and Animal Health (DGLAH 2015), Ministry of Agriculture. Data from CSA is published census data that have associated uncertainty estimates with variety within ±20%. Population of beef cattle included in the calculation was data from 2006 to 2014.



*LBW: Live body weight
**Feeding situation: Description of the extent to which cattle are stall fed, grazed or pastured over large geographical areas (IPCC 2006). This information is required to estimate the net energy expended by the animal during the acquisition of feed

Figure 1. Flow chart of methodology used for enteric methane EF estimate: IPCC (2006).

Compiled data were summarized by the two origin of local beef cattle and imported beef cattle. Local origin beef cattle production, which has more variation of live weight, was categorized by four sub-categories, namely weaning (0-1 year), yearling (1-2 years), young (2-4 years) and mature (more than 4 years). Imported beef cattle has short time living in Indonesia because of industrial meat production (aprroximately 120 days). Therefore, imported beef cattle was separated from local beef cattle as one of sub-category. Due to differences in the live weight for each sub-category, therefore each sub-category of beef cattle requires different value of EF. The sub-categories of beef cattle in Indonesia and composition of each sub-cetagory in total population are presented in Table 1.

Feeding situation and farming system for beef cattle in Indonesia

Data related to description of feed ingredients, consumption/intake, and gross energy value of feed ingredients was compiled from national scientific report, laboratory analysis and published papers. Animal's feeding situation is required to estimate the net energy expended by the animal during the acquisition of feed (IPCC 2006). Indonesia has many variations of feeding systems, which are depend on geographical areas and climate. Indonesia is archipelago country with 5 big islands from western to eastern part of Indonesia. Each part is determined by some agroecosystem zones.

The feeding situations of beef cattle are defined by analyzing farming system and feeding practices. In Indonesia, the farming systems for beef cattle are defined to intensive, semi intensive and extensive farming system, which are corresponded to feeding input and production. Most of small holder farmers apply intensive and semi intensive traditional farming systems, which are implement grass, native grass and agricultural by-product or crop-residues as sources of feed for beef cattle. While in intensive farming system as adopted by industrial farming, total mixed ration and concentrated feed are used to fed the imported beef cattle. Therefore, in the present study, the intensive feeding system is selected as the most representative for the major farming system in Indonesia.

Feed ingredients for local beef cattle are divided into three part, namely protein, energy and fibre source feeds. Fibre source feed includes cultivation grass, native grass, agricultural by-product such as rice straw, corn straw, oil palm leaves, sugar cane top. While energy source feed inlcludes rice brand, ground nut shell, palm kernel meal, pineaple processing waste, cassava industrial. Meanwhile for protein source feed includes legume leaves, cassava leaves. Industrial farming of beef cattle (feedlot) mostly uses total mixed ration to fed the animals (concentrate+fiber source feeds).

Feed and gross energy intake

Daily feed intake for each livestock sub-category was calculated based on dry matter intake (kg/day) and gross energy intake (MJ/day). Dry matter intake and digestibility of feed for each livestock sub-category, live weight of animals for each sub-category were collected from result of researchs and from published paper (Nurhayu et al. 2010; Nurhayu et al. 2011; Mathius 2010; Ratna et al. 2013; Soeharsono et al. 2010; Widyaningrum et al. 2013; Koddang 2008; Nugroho et al. 2015). Due to the large variations of feed available and offered to the animals in all provinces of Indonesia, the feeds were grouped into 5 types of feed for beef cattle sub-categories. The gross energy content of each type of feed was calculated from the average of laboratory analysis (Table 2).

Table 1. Sub-categories of beef cattle in Indonesia based on production level, live weight and each composition in the population

Sub-categories/age	Average live weight (kg)	Composition in total population (%)*
Weaning (0-1 year)	100	19.30
Yearling (1-2 year)	200	25.85
Young (2-4 year)	250	18.15
Mature (>4 year)	400	26.89
Imported (fattening)	350	9.81

*Source: DGLAH (2015)

Table 2. Type of feed and gross energy content used in the calculation

Type of feeds	Gross energy (MJ/kg DM)
Grass	17.4±0.540
Agricultural/plantation by-product	15.5±0.804
Leguminouse leaves	19.7±0.786
Grain/concentrate	20.2±0.754
Complete feed/commercial feed	18.9±0.886

Sources: Laboratory analyses

Developing enteric CH_4 EFs

Emision factor (EF) for enteric CH_4 fermentation was developed for each livestock sub-category based on gross energy intake. According to the guideline book of IPCC (2006), the equations to determine EFs for each sub-category spesific for Indonesia are:

Local beef cattle:

$$EF = (GEI \times (Ym/100) \times 365)/55.65$$

Imported beef cattle:

$$EF = (GEI \times (Ym/100) \times 120)/55.65$$

In this equation, EF is the emission factor (kg CH_4 head-1 yr-1), GEI is gross energy intake (MJ head-1 day-1), *Ym* is CH_4 conversion factor (%) of GEI in feed that is converted to CH_4, factor 55.65 (MJ/kg CH_4) is the energy content of methane (IPCC 2006). It is assumed that the EF was calculated for 365 days for local beef cattle for all sub-category, and only 120 days for imported beed cattle that going to feedlot system.

The CH_4 conversion factor (*Ym*) specific for Indonesia is still not available for all type of feeds. Therefore the *Ym* used is from default factor of IPCC (2006). A *Ym* of 3.0%±1.0% was used for imported beef cattle because concentrated feed are offered more than 90%. While, a *Ym* of 6.5%±1.0% was used for local beef cattle in Indonesia because the feed types primarily fed to the animals are low quality crop residues and by-products (rice brand, palm kernel meal, corn straw, rice straw, and others). The EF developed for local beef cattle was over a year (365 days), while for imported cattle was one periode of fedlot (120 days). Gross energy intake is filled in by real data for each livestock sub-category. The factor of 55.65 (MJ/kg CH_4) is the constanta indicates the energy content of the methane (IPCC 2006).

CH_4 emission from beef cattle

Once the EF values specific for Indonesia for all sub-categories of beef cattle were developed, they were used to estimate CH_4 emitted from beef cattle. Calculation of emission CH_4 from enteric fermentation from beef cattle in Indonesia is based on the beef cattle population or each sub-category and the value of EF specific for Indonesia, by using equation:

$$CH_4 \text{ emission} = EF_{(T)} \times (N_{(T)} / 10^6) \times 21/1000$$

In this equation, CH_4 emission is CH_4 emitted from enteric fermentation (Gg CO2-e/year), $EF_{(T)}$ is the *emission factor* for each sub-category of beef cattle (kg CH_4/head/year). The values of EF used are resulted from the calculation reported in this paper, $N_{(T)}$ is the population of beef cattle for each sub-category T in Indonesia (head), T is sub-category of beef cattle in Indonesia, 21/1000 is the convertion factor from CH_4 to CO_2-e (IPCC 2006). Population of beef cattle in each sub-category since year 2006 to 2014 is presented in Table 3.

Table 3. Population of beef cattle based on sub-category since 2016 to 2014

Sub-category	Population (x 000) (heads)								
	2006	2007	2008	2009	2010	2011	2012	2013	2014
Weaning (0-1 year)	2,099	2,222	2,366	2,463	2,621	2,861	3,084	2,448	2,842
Yearling (1-2 year)	2,811	2,977	3,168	3,298	3,511	3,832	4,131	3,279	3,807
Young (2-4 year)	1,974	2,090	2,225	2,316	2,465	2,691	2,900	2,303	2,673
Mature (>4 year)	2,924	3,096	3,296	3,431	3,652	3,986	4,297	3,411	3,960
Imported	1,067	1,130	1,202	1,252	1,332	1,454	1,568	1,245	1,445
Total	10,875	11,515	12,257	12,760	13,582	14,824	15,981	12,686	14,727

Sources: CSA (2015) and DGLAH (2015)

RESULT AND DISCUSSION

The values of EF for beef cattle in Indonesia

Methane EFs for all sub-categories of beef cattle calculated based on local data of gross energy intake are presented in Table 4.

Overall, enteric CH_4 EF for beef cattle in Indonesia is 33.14 kg/head/year. The value is averaging from the EF value of all sub-categories and their composition in the population. Imported cattle has CH_4 EF (25.49 kg/head/year) lower than local beef cattle due to short periode living in a year. The imported cattle also fed by high quality feed with more than 85% of concentrate in their ration in the form of complete feed. Live weight of cattle becomes a consideration on the amount of concentrate offered to the animals. In the first month of fattening period, the cattle were fed with lower level of concentrate (70-80% of total daily ration) than the cattle in the last period of fattening. The amount of concentrate offered was increased up to 90% as the body weight of cattle increased during the last month of fattening period. The fiber contained in the complete feed was lower than in convensional mixed feed (grass + legume + concentrate) fed to most of local beef cattle. In ruminant metabolism, dietary fiber is responsible for producing free H gas in the rumen. The free H is then used for CH_4 gas formation in the rumen.

The average enteric CH_4 EF for local beef cattle was 36.75 kg/head/year, due to the variation of feeds consumed (grass, agricultural waste and small amount concentrated feed) by the cattle. Because of the limmitation on the data available in the country for the methane conversion factor (*Ym*), therefore, calculation of EF values for the beef cattle in Indonesia used the *Ym* value as stated in the IPCC (2006). The *Ym* values available in the guideline are 3.0%±1%, for ration which is consisted of >90% concentrate, and 6.5%±1% for the ration that is consisted of high fibre diet. Local beef cattle in Indonesia mostly maintains in traditional farming system, where the feed offered consisted of 64.34% of forage and 35.66% of concentrate. With those such feed type, the *Ym* value used for the ration offered to local beef cattle is assumed to be closed to 6.5%±1%, according to the IPCC guideline. Imported beef cattle in fedlot system were fed by complete feed, which contains materials as sources of fibre and concentrate. With the variation of concentrate levels in the complete feed offered to the cattle at different time of fattening, the *Ym* value used in the calculation was 3%±1%, with the assumption that the value is fit with the type of feed offered to the imported cattle in fedlot system.

Local beef cattle in Indonesia was sub-categorized by age of animal, which determines their level of production. The proportion of mature local beef cattle (>2 years) is the highest (>50%) among other sub-categories, while the proportion of imported beef cattle is only 9.81%. With this such proportion, therefore, the EF value of all beef cattle in Indonesia would be more affected by local beef cattle than imported beef cattle.

Comparison of enteric CH_4 EF among some countries and default factor from IPCC (2006) are presented in Table 5.

There is a different value of enteric CH_4 EF between the default factor from IPCC (2006) and local Indonesia EF calculated by using Tier-2 method. On the whole, the enteric EF for all sub-categories of beef cattle in Indonesia (weaning, yearling, young, mature and imported) generated from this study (Table 4) are lower than the EF value from IPCC (2006). The Enteric CH_4 EF for beef cattle in Indonesia calculated using Tier-2 method is 36.75% lower than IPCC default factor for Tier-1 for Asia countries (IPCC 2006). In general, the differences indicated between enteric CH_4 EF generated by the present study and default enteric CH_4 EF from IPCC might be attributed to demographic profile and animal's growth performance data, in particular live weight and feeding system including the type of feed (IPCC 2006, annex 10A.1, Table 10A.2).

Table 4. Calculated gross energy intake (MJ/head/day) and enteric CH_4 EF (kg/head/year) for each sub-category of beef cattle in Indonesia

Sub-category	GEI* (MJ/head/day)	CH_4 EF (kg/head/year)	All beef cattle** (CH_4 EF kg/head/year)
Weaning (0-1 year) female + male	42.65±0.998	18.18±0.426	
Yearling (1-2 year) female + male	63.75±0.893	27.18±0.381	
Young (2-4 year) female + male	97.98±1.112	41.77±0.474	33.14±0.757
Mature (>4 year) female + male	131.11±4.632	55.89±1.975	
Imported (fattening) male	394.00±8.167	25.49±0.528	

* Calculated from daily feed intake and gross energy contents of the feeds
** Calculated based on the composition of each sub-category in the population

Table 5. Comparison between enteric CH_4 EF for Indonesia, default factor IPCC and other countries

Indonesia	IPCC (2006)	Other countries
Tier-2	Tier-1[e]	Tier-2 for beef cattle
Local cattle = 36.75	Other cattle (non dairy cattle)	Benin[a] : = 39.5
Imported cattle (Fedlot) = 25.49	for Asia region = 47	China[b] : MFF[c] = 48.3
All beef cattle = 33.14		MMF [c] = 57.5
		Young = 31.2
		Fedlot = 33.1[a]
		Malaysia[d] = 41.33

[a]) Kouazounde et al. 2015
[b]) USEPA (1994)
[c]) MFF : mature female farming; MMF: mature male farming
[d]) Hatton et al. 2013
[e]) IPCC (2006)

The lower enteric CH_4 EF of Indonesian beef cattle compared to IPCC default factor is suggested due to lower beef cattle live weight for each sub-category. For example, the live weight of mature beef cattle in Indonesia is averaged for about 400 kg (Widyaningrum et al. 2013), while the weight of animal for the same sub-category in IPCC guideline (2006) is 450 kg. The weight of imported beef cattle going to fedlot industry in Indonesia is averaged for about 350 kg, while in the IPCC guideline (2006), the weight of cattle in fedlot is 415 kg.

The default EF values suggested by IPCC guideline (2006) for Asia were averaged from many researchs in many Asia countries. Meanwhile, the EFs generated from this study includes a correction factor related to population sub-categorizing and different gross energy intake, which also separation between imported cattle and local cattle. The reduction value of CH_4 EF is about 21.8085% when the method was changing from Tier-1 (47 kg CH4/head/year, according to IPCC 2006) to Tier-2 (36.75 kg CH_4/head/year, generated from this study) for local beef cattle. The default value of EF for non dairy cattle (other cattle) in Asia region using Tier-1 as suggested by IPCC guideline (2006) is 47 kg CH_4/head/year, which is explained as small commercialised dairy sector.

The default EF for other cattle in the region of Asia with sub category mature (female and male) and maintain in stall fed is 50-59 kg CH_4/head/year (Tier-2, Table 10.A.2; page 10.74, IPCC 2006). The EF value for mature (female and male) for local beef cattle presented in this paper is 55.89 kg CH_4 $head^{-1}$ $year^{-1}$, which is still in the range of the default EFs IPCC. Althought there are some differences of the sub category between the two group. In Indonesia, the animal's weight for sub category of mature beef cattle is in the range of 400-450 kg, while the mature sub category in the IPCC has live weight of 350 up to 450 kg.

Moreover, the EF value for sub-category young cattle, which is maintain in pasture system, is 36 kg CH_4/head/year (Table 10.A.2; page 10.74, IPCC 2006). This EF value is lower than the value of EF presented in this paper for sub-category young maintained in stall fed system (41.77 kg CH_4/head/year). According to the live weigh of animals, the sub-category young in the IPCC guideline (Table 10.A.2; page 10.74) has live weigh of 200 kg. This live weight is similar with the weight of animals in sub category yearling (200 kg), with the EF value is 27.18 kg CH_4/head/year. Thus the sub-category of young in the IPCC is similar with the sub-category yearling in local beef cattle presented in this paper.

The EF value for beef cattle also is 6.962% lower than the EF value for beef cattle in Benin (Kouazounde et al. 2015) and 19.5137 % lower than those EF in China (USEPA 1994). Even with Malaysia, the neighbor country with Indonesia, the local enteric CH_4 EF is 22.94% less than those of EFs for Malaysia 's cattle (Hatton et al. 2013). Each country has local specific type of feeds and type of beef cattle breed, which then produce different enteric CH_4 profile. Since CH_4 is formed during feed fermentation in the rumen, thus differences in the type of feed consumed by the animals would result in different CH_4 production. Differences between the value of EF in Indonesia and other countries could be related to differences in the population sub-category and feeding situation. Hence, the estimation of enteric CH_4 EF is country specific factor, which is always changing to suit with any upgrading data as key factors in calculation. The study reveals the national average EF for beef cattle from Indonesia is consistent with other reports for other cattle from Asia being lower than this author finding because of upgrading method of calculation from Tier-1 to Tier-2 methods.

Estimation of methane emission from beef cattle by using Tier-2 method

Enteric CH_4 EF from beef cattle in Indonesia generated from this study is then used to estimate total emission of enteric CH_4 from beef cattle. The calculation used beef cattle population as shown in Table 3 and the value of EF generated from this study as presented in Table 4. The results of those calculation are presented in Table 6.

The total enteric CH_4 emission from beef cattle increases 4.41.% per year from 2006-2014 due to increasing in population of beef cattle in Indonesia. However, there is a decreasing in the enteric CH_4 emission from beef cattle in 2012 to 2013. This reduction is due to a decreasing in beef cattle population as a result of government's policy about limitation in importing the beef cattle. In the other side, the local beef cattle population also decreased at the time because there is an increasing in animal protein consumption (CSA 2015). However, the trend of CH_4 emission increases year by year (Figure 2) as a result of an increasing in the population of beef cattle.

Imported cattle, which represents the fedlot industry, only contributes for about 7.85% of total enteric CH_4 emission from beef cattle, due to their small population compared to the local beef cattle. Other reason is the imported beef cattle uses good quality feed (high concentrate fed) which produces low methane per unit dry matter of feed digested (Migwi et al. 2011a; Migwi et al. 2011b; Pedreira et al. 2013; Beauchemin & McGinn 2005).

There is a different value on the national enteric CH_4 emission from beef cattle when they are calculated by using local EF generated from this study (Tier-2 method) compared to those were calculated by using default EF from IPCC (Tier-1 method). The comparisson of methane emission from beef cattle calculated by using Tier-1 method (Widiawati 2013) with those calculated by using Tier-2 method generated from this study is shown in Figure 3. There is a reduction in the national CH_4 emission from beef cattle by 32.2% up to 45.5% when they are calculated by using Tier-2 method, during the period of year 2006 to year 2014.

Table 6. Estimation of enteric CH_4 emission from beef cattle in Indonesia from 2006 to 2014 calculated by using Tier-2 method

Sub-category	Methane from enteric fermentation (Gg CO_2-e/year)								
	2006	2007	2008	2009	2010	2011	2012	2013	2014
Weaning (0-1 year)	0.801	0.849	0.903	0.940	1.001	1.093	1.178	0.935	1.085
Yearling (1-2 year)	1.604	1.699	1.808	1.883	2.004	2.187	2.358	1.872	2.173
Young (2-4 year)	1.732	1.833	1.951	2.032	2.162	2.360	2.544	2.020	2.345
Mature (>4 year)	3.432	3.634	3.868	4.028	4.287	4.679	5.044	4.0043	3.474
Imported (fattening)	0.571	0.605	0.644	0.670	0.713	0.778	0.839	0.666	0.773
Total	7.274	7.702	8.198	8.534	9.084	9.915	10.689	8.485	9.850

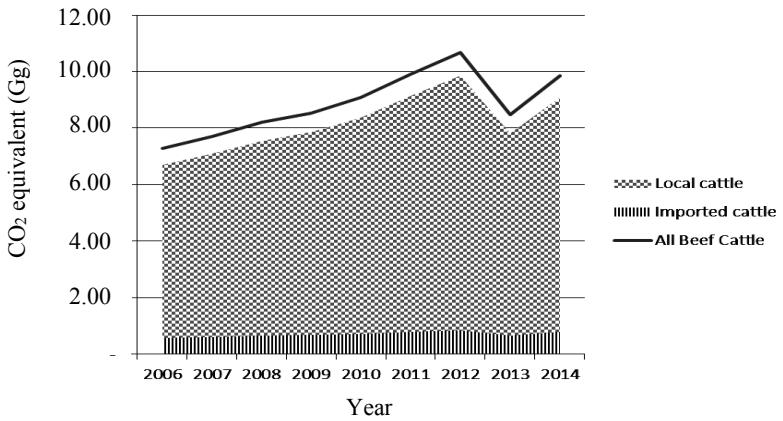


Figure 2. The trend of enteric CH_4 emission from beef cattle in Indonesia since year 2006 to year 2014, calculated by using Tier-2 method.

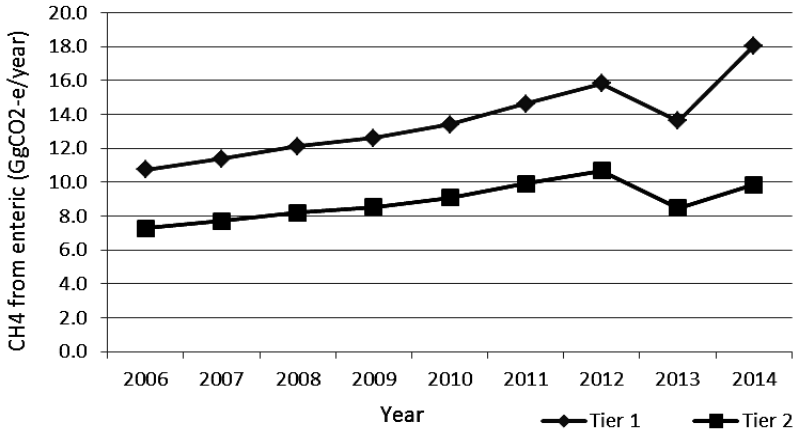


Figure 3. The comparison between national enteric CH_4 emission from beef cattle calculated by using Tier-1 and Tier-2 methods.

Reduction of enteric CH_4 emission from beef cattle occured when the calculation was changed from Tier-1 to Tier-2 method. In Tier-2 method, simple technologies in feeding management have been addressed. Most of the feeding managements adopted by farmers are supplementation of feed, by using leguminous leaves or concentrate. These two types of feed supplement increase the quality of basal feed, which also have indirect effect on mitigate the production of CH_4 during rumen fermentation (Martin et al. 2010; Cottle et al. 2011; Patra 2012; Jayanegara et al. 2009; Jayanegara et al. 2011; Holtshausen et al. 2009).

The national average EF for beef cattle generated from this study is representing the EF for all beef cattle in all provinces of Indonesia. The application of the Tier-2 method suggested in IPCC (2006) to Indonesian conditions illustrates the challenges in developing country-specific EFs stemming from lack of data within provinces in Indonesia, where the type of feeds are vary. It is also become a further challange since there is a variation of beef cattle breed among the Indonesia regions. Further research therefore should focus on the estimation of uncertainties in Tier-2 inputs. The calculation must be further developed for different breed of beef cattle as well as different feeding systems (intensive, extensive, pasture).

CONCLUSION

The value of EF for all Indonesia's beef cattle calculated by using Tier-2 method is 33.14 kg CH_4/head/year. The value is lower than the default EF suggested by IPCC for Asia country (47 kg CH_4/head/year). The national methane emission from beef cattle estimated by using Indonesia's EF for beef cattle is lower than those estimated by using EF value suggested by IPCC (Tier-1 method). The EF value generated from this study represents the Indonesia's specific livestock system.

ACKNOWLEDGEMENT

The authors have benefited from the support of Dr. Harry Clark (NZ-AgResearch). We have also benefited from the tim-work and collaboration within the member of national Tier-2 team, Prof. Dr. Agung Purnomoadi (Diponegoro University); Noorhuda Krisna, SPt., MSi (BCRS); Dr. Yeni N. Anggraeni (BCRS); Dr. Simon P. Ginting (GRS); Dr. Antonius (GRS), Dr. Dwi Yulistiani (IRIAP); Dr. Wisri Puastuti (IRIAP); Rantan Krisnan, SPt., MSi (IRIAP); Cecep Hidayat, SPt., MSi (IRIAP); Teguh Wahyono, SPt., MSi (NNEAI) and Ir. Ali Pramono, MSi (IRIAE).

REFERENCES

Beauchemin KA, McGinn SM. 2005. Methane emissions from feedlot cattle fed barley or corn diets. J Anim Sci. 83:653-661. doi: 10.2527/2005.833653x.

[CSA] Central Statistic Agency. 2015. Statistik Indonesia 2015. Jakarta (Indones): Central Statistic Agency.

Cottle DJ, Nolan JV, Wiedemann SG. 2011. Ruminant enteric methane mitigation: a review. Anim Prod Sci. 51:491-514. doi: 10.1071/AN10163.

Dijkstra J, Oenema O, Bannink A. 2011. Dietary strategies to reducing N excretion from cattle: implications for methane emissions. Curr Opin Environ Sustain. 3:414-422. doi: 10.1016/j.cosust.2011.07.008.

[DGLAH] Directorate General of Livestock and Animal Health. 2015. Statistik peternakan dan kesehatan hewan. Jakarta (Indones): Directorate General of Livestock and Animal Health, Ministry of Agriculture.

Gerber P, Hristov A, Henderson B, Makkar H, Lee JOC, Meinen R, Montes F, Ott T, Firkins J. 2013. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: A review. Animal 7:220-234. doi: 10.1017/S1751731113000876.

Gregorini P, Beukes P, Bryant R, Romera A. 2010. A brief overview and simulation of the effects of some feeding strategies on nitrogen excretion and enteric methane emission from grazing dairy cows. In: Edwards GR, Bryant R, editors. Proceedings of the 4th Australasian Dairy Science Symposium. Lincoln (Aust): Caxton Press. p. 29-43.

Hammond KJ, Burke JL, Koolaard JP, Muetzel S, Pinares-Patino CS, Waghorn GC. 2013. The effect of feed intake on enteric methane emissions from sheep fed fresh white clover (*Trifoliumrepens*) and perennial ryegrass (*Loliumperenne*) forages. Anim Feed Sci Technol. 179:121-132. doi: 10.1016/j.anifeedsci. 2012.11.004.

Hatton V, Kinh LV, Boonyanuwat K, Shanmugavelu S, Widiawati Y. 2013. Livestock greenhouse gas emissions from four countries in South East Asia: A collaborative approach to a regional problem. Proceedings of the 5th Greenhouse Gasses and Animal Agriculture Conference. Dublin (Irel). p. 587

Holtshausen L, Chaves AV, Beauchemin KA, McGinn SM, McAllister TA, Odongo NE, Cheeke PR, Benchaar C. 2009. Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. J Dairy Sci. 92:2809-2821.

[IPCC] Intergovernmental Panel on Climate Change. 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme. Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K, editors. Kanalawa (Jpn): Institute for Global Environmental Strategies.

Jayanegara A, Togtokhbayar N, Makkar HPS, Becker K. 2009. Tannins determined by various methods as predictors of methane production reduction potential of plants by an *in vitro* rumen fermentation system. Anim Feed Sci Technol. 150:230-237. doi: 10.1016/j.anifeedsci.2008.10.011.

Jayanegara A, Wina E, Soliva CR, Marquardt S, Kreuzer M, Leiber F. 2011. Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. Anim Feed Sci Technol. 163:231-243. doi: 10.1016/j.anifeedsci.2010.11.009.

Koddang MYA. 2008. Pengaruh tingkat pemberian konsentrat terhadap daya cerna bahan kering dan protein kasar ransum pada sapi Bali jantan yang mendapatkan rumput Raja. J Agrol. 15:343-348.

Kouazounde JB, Gbenou JD, Babatounde S, Srivastava N, Eggleston SH, Antwi C, Baah J, McAllister TA. 2015. Development of methane emission factors for enteric fermenation in cattle from Benin using IPCC Tier 2 methodology. Animal. 9:526-533. doi: 10.1017/S1751731114002626.

Lassey KR. 2007. Livestock methane emission: from the individual grazing animal through national inventories to the global methane cycle. Agric Forest Meteorol. 142:120-132. doi: 10.1016/j.agrformet.2006.03.028.

Martin C, Doreau M, Morgavi DP. 2010. Methane mitigation in ruminants: from rumen microbes to the animal. Animal. 4:351-365

Mathius I-W. 2010. Optimalisasi pemanfaatan bungkil inti sawit untuk sapi yang diberi pakan dasar rumput alam. In: Prasetyo LH, Natalia L, Iskandar S, Puastuti W, Herawati T, Nurhayati, Anggraeni A, Dharmayannti NLPI, Estuningsih SE, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indonesia): Indonesian Center for Animal Research and Development. p. 161–169.

Migwi PK, Godwin I, Nolan JV, Khan LP. 2011a. The effect of energy supplementation on intake and utilization efficiency of urea-treated low-quality roughage in sheep. I. Rumen digestion and feed intake. Asian-Australas J Anim Sci. 24:623-635. doi: 10.5713/ajas.2011.10282.

Migwi PK, Godwin I, Nolan JV. 2011b. The effect of energy supplementation on intake and utilization efficiency of urea-treated low-quality roughage in sheep. II. Rumen kinetics and acetate clearance rate. Asian-Australas J Anim Sci. 24:774-780. doi: 10.5713/ajas.2011. 10283.

Nugroho HD, Permana IG, Despal. 2015. Utilization of bioslurry on maize hydroponic fodder as a corn silage supplement on nutrient digestibility and milk production of dairy cows. J Anim Sci Technol. 38:70-76.

Nurhayu A, Pasambe D, Sariubang M. 2010. Kajian pemanfaataan pakan lokal dan UMB untuk penggemukan sapi potong di kab Pinrang, Sulsel. In: Prasetyo LH, Natalia L, Iskandar S, Puastuti W, Herawati T, Nurhayati, Anggraeni A, NlPI D, Estuningsih SE, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 194-199

Nurhayu A, Sariubang M, Nasrullah, Ella A. 2011. Respon Pemberian Pakan Lokal terhadap produktivitas sapi Bali dara di Kab Bantaeng. In: Prasetyo LH, Damayanti R, Iskandar S, Herawati T, Priyanto D, Puastuti W, Anggraeni A, Tarigan S, Wardhana AH, Darmayanti NLPI, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 115-120.

Patra AK. 2012. Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. Environ Monit Assess. 184:1929-1952. doi: 10.1007/s10661-011-2090-y.

Pedreira MdS, Oliveira SGd, Primavesi O, Lima MAd, Frighetto RTS, Berchielli TT. 2013. Methane emissions and estimates of ruminal fermentation parameters in beef cattle fed different dietary concentrate levels. R Bras Zootech. 42:592-598. doi: 10.1590/S1516-35982013000800009.

Ratna AD, Hendri Y, Bamualim A. 2013. Respon pertumbuhan sapi Simmental yang diberi pakan hasil ikutan industri sawit di Sumbar. In: Purwantari ND, Saepulloh M, Anggraeni A, Ginting S, Priyanti A, Wiedosari E, Yulistiani D, Inounu I, Bahri S, Puastuti W, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 162-168.

Rong-Zhen Z, Fang Y, Sun HX, Wang M, Zou DW. 2016. Rumen methane output and fermentation characteristics of gramineous forage and leguminous forage at differing harvest dates determined using an in vitro gas production technique. J Integr Agric. 15:414-423. doi: 10.1016/S2095-3119(15)61036-X.

Soeharsono, Saptati RA, Diwyanto K. 2010. Penggemukan sapi lokal hasil IB dan sapi bakalan impor dengan menggunakan bahan pakan lokal. In: Prasetyo LH, Natalia L, Iskandar S, Puastuti W, Herawati T, Nurhayati, Anggraeni A, Darmayanti NLPI, Estuningsih SE, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 116-122.

[USEPA] United States of Environmental Protection Agency. 1994. International Anthropogenic Methane Emissions: Estimates for 1990. EPA-230-R-93-010. Washington DC (USA): United States of Environmental Protection Agency.

Widiawati Y. 2013. Estimation of Methane Emission from Enteric Fermentation and Manure Management of Domestic Livestock in Indonesia. Proceedings of the 5th Greenhouse Gasses and Animal Agriculture Conference (GGAA 2013). Dublin (Irel). p. 443.

Widyaningrum Y, Pamungkas D, Kote M, Mariyono. 2013. Pertumbuhan sapi Bali jantan muda pada agroekosistem lahan kering iklim kering Nusa Tenggara Timur. In: Purwantari ND, Saepulloh M, Anggraeni A, Ginting S, Priyanti A, Wiedosari E, Yulistiani D, Inounu I, Bahri S, Puastuti W, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 276-281.

Greenhouse Gas Emissions from Cattle Production Sector in South Korea

Febrisiantosa A[1,2], Lee JH[1], Choi HL[1,2]

[1]*Department of Agricultural Biotechnology and Research Institute for Agricultural and Life Science, Seoul National University, Seoul, Republic of Korea, 151-742.*
[2]*Research Unit for Natural Product Technology, Indonesian Institute of Sciences, Jl. Jogja Wonosari km 32, Gading Playen, Gunungkidul, Yogyakarta, Indonesia*
[2]*Resourcification Research Center for Crop-Animal Farming (ReCAF), Gwanak-gu, Seoul. S. Korea*
E-mail: andi82@snu.ac.kr

(received 22-04-2016; revised 10-05-2016; accepted 29-06-2016)

ABSTRAK

Febrisiantosa A, Lee JH, Choi HL. 2016. Emisi gas rumah kaca dari sektor produksi ternak di Korea Selatan. JITV 21(2): 112-123. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1359

Negara Korea Selatan menyatakan akan mengurangi emisi gas rumah kaca sebanyak 30% dari level emisi saat ini pada tahun 2020. Penelitian ini dilakukan untuk mengevaluasi emisi gas rumah kaca dari sektor produksi sapi potong di Korea Selatan. Emisi gas rumah kaca dari aktivitas produksi sapi perah, sapi lokal Non-Korea dan sapi lokal Korea (Hanwoo) di 16 provinsi Korea Selatan selama sepuluh tahun (2005-2014) diestimasikan menggunakan metode khusus berdasarkan Guidelines for National Greenhouse Gas Inventory of the IPCC (2006). Emisi yang dievaluasi dalam penelitian ini meliputi gas metan dari fermentasi enterik, gas metan dari pengelolaan kotoran, oksida nitrat dari pengelolaan kotoran dan karbon dioksida dari penggunaan energi secara langsung. Lebih dari 10 tahun terakhir, aktivitas produksi sapi Hanwoo merupakan penyumbang utama (83.52% dari total emisi sektor produksi ternak sapi) CH_4 dari fermentasi enteric, CH_4 dari pengelolaan kotoran, NO_2 dari pengelolaan kotoran dan NO_2 dari penggunaan energi secara langsung pada sektor peternakan sapi di Korea Selatan.

Kata Kunci: Metan, Oksida Nitrat, Karbondioksida, Sapi, Pemanasan Global

ABSTRACT

Febrisiantosa A, Lee JH, Choi HL. 2016. Greenhouse gas emissions from cattle production sector in South Korea. JITV 21(2): 112-123. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1359

South Korea has declared to reduce greenhouse gas emissions by 30% compared to the current level by the year 2020. The greenhouse gas emissions from the cattle production sector in South Korea were evaluated in this study. The greenhouse gas emissions of dairy cattle, Non-Korean native cattle and Korean native (Hanwoo) cattle production activities in 16 local administrative provinces of South Korea over a ten-year period (2005–2014) were estimated using the methodology specified by the Guidelines for National Greenhouse Gas Inventory of the IPCC (2006). The emissions studied herein included methane from enteric fermentation, methane from manure management, nitrous oxide from manure management and carbon dioxide from direct on-farm energy use. Over the last ten years, Hanwoo cattle production activities were the primary contributor of CH_4 from enteric fermentation, CH_4 from manure management, NO_2 from manure management and CO_2 from on-farm energy use in the cattle livestock sector of South Korea, which comprised to 83.52% of total emissions from cattle production sector.

Key Words: Methane, Nitrous Oxide, Carbon Dioxide, Cattle, Global Warming

INTRODUCTION

Greenhouse gas (GHG) emissions from human activities become a focus of worldwide attention because of global warming issues. With global warming, the equilibrium of ecosystems is threatened by climate change. The agricultural sector contributes 18% (7.1 billion tonnes CO_2 equivalent) to the total global greenhouse gas emissions (FAO 2006). Although agricultural activity contributes only 9% to global CO_2 emissions, agriculture generates 65% of human-related nitrous oxide (N_2O) and 35% of methane (CH_4) with global warming potentials (GWPs) of 298-fold and 25-fold that of CO_2, respectively (USEPA 2014; IPCC 2006; Smith et al. 2007). South Korea pledged to reduce greenhouse gas emissions by 30% below the current levels by 2020 or by 4% below the levels of 2005 (UNFCCC 2011; Gerber et al. 2013). Livestock production is a critical contributor in agricultural activity that produces greenhouse gases and contributes approximately 42% to the total GHG emissions, with 28% of the emissions directly from enteric fermentation and 14% from indirect emissions due to the handling, storage, and land application of manure (AAFCCT 2000).

Production of cattle is an important part of economic growth of the livestock industry as it meets the increasing demand of meat and milk products. However, along the production, greenhouse gases are emitted. Few studies have estimated GHG emissions from livestock sector that follow the IPCC guidelines. Merino et al. (2011) inventoried the regional methane and nitrous oxide emissions from ruminant livestock in Basque country, and Patra (2014) studied the trends and projected estimation of GHG emissions from Indian livestock in comparison with the global GHG emissions and those from developing countries. In South Korea, Ji & Park (2012) found that the annual growth rates of enteric CH_4 emissions and CH_4 and N_2O emissions from manure management from 1990 to 2009 were 1.7%, 2.6% and 3.2%, respectively. Lee & Lee (2003) investigated national methane emissions from the enteric fermentation of livestock, and Jo et al. (2015) estimated methane emissions factor from enteric fermentation of Hanwoo steers. Amon et al. (2006) estimated GHG emissions from different management system of dairy cattle. In South Korea, Korean native (Hanwoo) cattle population is already be distinguished from the other beef producing cattle breed population. Other source of beef production is the dairy cattle steers which is categorized as Non-Korean native cattle. However, the information has not been obtained for recent GHG emissions from cattle production activities in South Korea. Therefore, the aim of this study was to estimate the emissions of greenhouse gases from the cattle production sector, including estimates for on-farm energy use, in South Korea.

MATERIALS AND METHODS

Scope and activity data

This study focused on three primary emission sources in cattle production activities: Emissions from enteric fermentation, emissions from manure management, and emissions from direct on-farm energy use. Methane was emitted from enteric fermentation and manure management, nitrous oxide from manure management and carbon dioxide from on-farm energy use. Data of livestock populations in South Korea were adopted from the Korean Statistical Information Services. Populations of cattle were based on the average of each annual quartile. Cattle population data between 2005 and 2014 are shown in Table 1. The cattle were divided into three categories (breed, age and sex). The breed category was divided into three subcategories (Hanwoo, beef, and dairy cattle), the age category was divided into three subcategories (under 1 y, 1-2 y, and over 2 y), and the sex was divided into two subcategories (male and female), except for the dairy cattle. The population estimates are presented for 16 local administrative provinces in South Korea.

Estimation of greenhouse gas emissions

Current assessment used a methodology specified in the Guidelines for National Greenhouse Gas Inventory (IPCC 2006) and the Greenhouse gas emissions from ruminant supply chains (Opio et al. 2013). The emissions were assessed in 16 local administrative provinces of South Korea. Global Warming Potentials (GWPs), based on the 4th Assessment Report of the IPCC (IPCC 2006), were used to convert N_2O and CH_4 values to CO_2-eq values. Consequently, the GWPs of 25 and 298 were used for CH_4 and N_2O, respectively.

Approaches used to estimate the emissions from cattle production were Tier-1 and Tier-2 methods in the IPCC guidelines. In this study, the different value of enteric fermentation factor was used for Hanwoo and Non-Korean native cattle since Korea has different category of beef production cattle. Default emission factors for the Tier-1 method were provided by the IPCC (2006). For some approaches using Tier-2

Table 1. Cattle population in South Korea (2005-2014)

Year	Korean native (Hanwoo) cattle (heads)	Non-Korean native cattle (heads)	Dairy cattle (heads)
2005	1,582,446	181,301	487,882
2006	1,781,256	177,743	471,214
2007	1,990,379	170,248	456,387
2008	2,232,247	165,220	446,319
2009	2,432,454	154,976	442,373
2010	2,709,201	157,362	435,048
2011	2,839,829	142,216	401,628
2012	2,937,828	124,854	412,828
2013	2,884,541	113,318	421,135
2014	2,724,879	92,275	427,782

Table 2. Approaches to estimate GHG emissions from cattle production in South Korea (2005-2014)

Emission sector	Korean native (Hanwoo) cattle	Non-Korean native cattle	Dairy cattle
CH_4 emissions from enteric fermentation	Tier-2	Tier-2	Tier-2
CH_4 emissions from manure management	Tier-2	Tier-2	Tier-1
N_2O emissions from manure management	Tier-1	Tier-1	Tier-1
CO_2 emissions from direct on-farm energy use for livestock	Tier-1	Tier-1	Tier-1

method, country-specific information was required. The estimations of emission factors for the Tier-2 method were adopted from the IPCC guidelines. Characteristics of the approaches used to estimate GHG emissions for cattle production are shown in Table 2. The estimation was conducted in 3 steps. In step 1, cattle population was classified into subgroups and each subgroup was characterized. In step 2, emission factor was estimated for each subgroup in kilograms of gas emission per animal per year.

In step 3, to estimate the emissions for the different subgroups, the emission factors of each subgroup were multiplied by the population size of that subgroup; the emissions of the subgroups were then summed to estimate total emission.

RESULTS AND DISCUSSION

CH_4 emission from enteric fermentation

Cattle consume organic matter that is degraded by rumen microbes with the production of CH_4 as a final product of metabolism; thus, enteric fermentation in cattle is a source of CH_4 emission. Tier-2 method was used in this estimation. Tier-2 methodology is more appropriate than the Tier-1 (Höglund-Isaksson 2012). Emission factors for methane enteric fermentation from cattle in South Korea are provided in Table 3, which was used to estimate enteric methane emission.

CH_4 emissions from cattle enteric fermentation of South Korea are shown in Table 4. Annual CH_4 emissions from the enteric fermentation of dairy cattle in the 16 provinces of South Korea between 2005 and 2014 decreased about 17.41%. However, it starts to increase after 2011. The highest CH_4 emissions from dairy cattle enteric fermentation was recorded in Gyeonggi. In seven of the provinces, the CH_4 emissions from dairy cattle enteric fermentation decreased in 2011 but then increased in 2014, compared to the emission in 2011. The local administrative provinces with decreased emission from dairy cattle enteric fermentation in 2014 compared to that in 2005 was Daegu (-56%), Daejeon (-100%) and Jeju (-18%).

The local administrative province with increased CH_4 emissions from dairy cattle enteric fermentation in 2014 compared to that in 2005 was Chungcheongnam (+8%). From enteric fermentation, dairy cattle emitted 1.19 Mt CO_2-eq in 2001 (Lee & Lee 2003), whereas in this study, dairy cattle emitted 1.21 Mt CO_2-eq in 2014. The total methane emissions from enteric fermentation did not increase significantly because the population remained constant during the last ten years.

Table 3. Emission factors to estimate methane emissions from enteric fermentation of cattle in South Korea

Cattle	Emission factor (kg CH_4/head/year)		
Korean native (Hanwoo)	under 1 year	1~2 years	2 years and over
Male	36.2	71.5	76.1
Female	34.3	63.6	73.1
Non-Korean native	under 1 year	1~2 years	2 years and over
Male	51.5	69.1	98.3
Female	23.0	50.7	63.5
Dairy	under 1 year	1~2 years	2 years and over
	41.24	53.55	53.40

Table 4. CH_4 emissions from the enteric fermentation of cattle in South Korea (2005-2014)

Year	Korean native (Hanwoo) cattle (Mt CO_2-eq/year)	Non-Korean native cattle (Mt CO_2-eq/year)	Dairy cattle (Mt CO_2-eq/year)	Total (Mt CO_2-eq/year)
2005	2.33	0.26	1.33	3.91
2006	2.64	0.25	1.27	4.17
2007	2.96	0.25	1.24	4.44
2008	3.35	0.24	1.20	4.79
2009	3.67	0.23	1.19	5.09
2010	4.10	0.23	1.18	5.51
2011	4.31	0.22	1.09	5.62
2012	4.47	0.19	1.16	5.82
2013	4.44	0.18	1.19	5.80
2014	4.21	0.14	1.21	5.57

Between 2005 and 2014, CH_4 emissions from the enteric fermentation of Non-Korean native cattle in South Korea decreased only about 0.46% or about 0.12 Mt CO2-eq. In all 16 provinces except for Busan, less CH_4 was emitted from Non-Korean native cattle enteric fermentation in 2005 compared to that in 2014. The largest CH_4 emissions decreasing between 2005 to 2014 was from Non-Korean native cattle in Chungcheongbuk with a decrease of 0.019 CO2-eq or about 52% compare to the initial. Local province with an increase of CH_4 emissions from Non-Korean native cattle enteric fermentation was Busan that increased the emission by about 10% during the last ten years. From the enteric fermentation of Hanwoo cattle in South Korea, annual CH_4 emissions increased by about 91.6% between 2005 and 2012 then decreased by about 5.8% in 2014. Compared to the emissions in 2005, all 16 local administrative provinces showed higher CH_4 emissions from the enteric fermentation of Hanwoo cattle in 2014. Among the 16 local administrativeprovinces in 2014, Gyeongsangbuk recorded as a province with highest CH_4 emissions from Hanwoo cattle enteric fermentation approximately by 0.91 Mt CO_2-eq. For the enteric fermentation of Hanwoo cattle between 2005 and 2014, local administrative provinces with an increase in the rate of CH_4 emissions above 100% were Incheon (+22,560.75 Mt CO_2-eq, +258%), Gyeonggi (+190.125 Mt CO_2-eq, +119%), and Jeollabuk (+272,742.75 Mt CO_2-eq, +117%). Lee & Lee (2003) reported that methane emission from the enteric fermentation of Hanwoo cattle was 1.54 Mt in 2001, whereas we found that methane emissions were 4.21 Mt CO_2-eq in 2014 from the enteric fermentation of Hanwoo. The largest contribution of methane from enteric fermentation was from Hanwoo cattle because of the highest population growth rate during ten-year period. Johnson & Johnson (1995) reported that many factors influence methane emissions from cattle, including the level of feed intake, type of carbohydrate in the diet, feed processing, addition of lipids or ionophores to the diet, and alterations in the ruminal microflora. Furthermore, the results of this study (4.21 Mt CO_2-eq from 2,724,879 head of Hanwoo cattle in a year) were consistent with those reported by Basarab et al. (2005), who reported that 8.34 Mt CO_2-eq were emitted from the enteric fermentation of 6,474,350 head of Non-Korean native cattle in a year (using a Tier-2 method).

CH_4 emission from manure management

Emissions of nitrous oxide from the management of Non-Korean native cattle manure were direct and indirect. Because the available data were limited for each variable, this assessment used Tier-1 method to estimate nitrous oxide emission. Annual average N excretion per head per year (Nex) value were used to estimate direct N_2O emission. The fraction of total annual nitrogen excretion for livestock and the emission factor value that were used in this assessment were 1 and 0.006 N_2O-N/kg N, respectively. These values were from the default values of the IPCC (2006). Methane emission factor is provided in Table 5 for the estimation of emission from the manure management of Non-Korean native cattle in South Korea. To evaluate the contribution of cattle production sector in South Korea to global warming, the global warming potentials of the GHG emissions were converted into units of Mt CO_2-eq.

CH_4 emission from manure management of cattle in South Korea between 2005 and 2014 are presented in Table 6. CH_4 emissio from manure management of dairy cattle decreased by about 9.46% in between 2005 to 2014. However, the lowest emission occurred in 2011 (0.0822 Mt CO_2-eq), with a slight increase

Table 5. Emission factors to estimate methane emissions from manure management of Non-Korean native cattle in South Korea

Cattle	Emission factor (kg CH_4/head/year)		
Hanwoo	under 1 year	1~2 years	Over 2 years
Male	0.60	1.01	0.94
Female	0.57	0.90	0.91
Beef	under 1 year	1~2 years	Over 2 years
Male	0.86	0.97	1.22
Female	1.10	1.32	1.62
Dairy	under 1 year	1~2 years	Over 2 years
	0.99	1.16	2.53

thereafter. Gyeonggi was the local administrative province with the highest CH_4 emissions (8.176 kt CO_2-eq) from manure management in 2014. About 15 local administrative provinces showed a slight decrease in CH_4 emissions from dairy cattle manure management with an annual growth rate of -1.02%, and one local administrative province (Chungcheongnam) showed an increase from 3.76 kt CO_2-eq in 2005 to 4.05 kt CO_2-eq in 2014.

Similarly, CH_4 emission from manure management of Non-Korean native cattle in South Korea between 2005 and 2014 decreased approximately 49% with an annual growth rate of -7.16% (Table 6). All the 16 local administrative provinces showed a decrease in CH_4 emission from Non-Korean native cattle manure management between 2005 and 2014. Gyeonggi was the local administrative province with the highest emissions of approximately 0.872 kt CO2-eq fromcattle manure management in 2014 among the 16 local administrative provinces.

CH_4 emission from manure management of Hanwoo cattle in South Korea increased between 2005 and 2014 by about 0.0252 MtCO_2-eq or 78%. Compared to the other local administrative districts, Gyeongsangbuk showed the highest CH_4 emissions from the manure management of Hanwoo cattle between 2005 and 2014 (12.32 kt CO_2-eq). The local administrative provinces with increased emissions of CH_4 above 100% from the manure management of Hanwoo cattle between 2005 and 2014 were Incheon (+39 kt CO_2-eq, +283.89%), Gyeonggi (+322.51 kt CO_2-eq +127.74%), Jeollabuk (+458.99 ktCO_2-eq, +122.92%), and Jeju (+39.80 ktCO_2-eq, +122.92%). Annual growth rate for CH_4 emission was 8.33% from manure management of Hanwoo cattle in South Korea between 2005 and 2014. Among the three types of cattle production in South Korea, methane emission increased only from the manure management of Hanwoo cattle.

N_2O emission from manure management

N_2O emissions from manure management of cattle are shown in Table 7. Emission of N_2O during manure treatment occurs in two forms, direct and indirect. Total

Table 6. CH_4 emission from the manure management of cattle in South Korea (2005-2014)

Year	Korean native (Hanwoo) cattle (Mt CO_2-eq/year)	Non Korean native cattle (Mt CO_2-eq/year)	Dairy cattle (Mt CO_2-eq/year)	Total (Mt CO2-eq/year)
2005	0.0320	0.0044	0.0243	0.0607
2006	0.0361	0.0044	0.0234	0.0639
2007	0.0405	0.0042	0.0226	0.0673
2008	0.0457	0.0040	0.0220	0.0718
2009	0.0501	0.0037	0.0218	0.0757
2010	0.0559	0.0038	0.0215	0.0813
2011	0.0587	0.0035	0.0201	0.0822
2012	0.0609	0.0030	0.0211	0.0851
2013	0.0605	0.0027	0.0216	0.0848
2014	0.0572	0.0022	0.0220	0.0815

Table 7. N_2O emissions from the manure management of cattle in South Korea (2005-2014)

Year	Korean native (Hanwoo) cattle (Mt CO_2-eq/year)	Non Korean native cattle (Mt CO_2-eq/year)	Dairy cattle (Mt CO_2-eq/year)	Total (Mt CO2-eq/year)
2005	0.3112	0.0541	0.1440	0.5093
2006	0.3503	0.0530	0.1391	0.5424
2007	0.3914	0.0508	0.1347	0.5769
2008	0.4390	0.0493	0.1318	0.6200
2009	0.4784	0.0462	0.1306	0.6552
2010	0.5328	0.0469	0.1284	0.7081
2011	0.5585	0.0424	0.1186	0.7195
2012	0.5777	0.0372	0.1219	0.7369
2013	0.5673	0.0338	0.1243	0.7254
2014	0.5359	0.0275	0.1263	0.6897

N_2O emission from manure management of dairy cattle between 2005 and 2014 decreased by about 14%. The lowest N_2O emission from dairy cattle manure management occurred in 2011, which increased thereafter. The largest decrease in percent emission was in Daejeon (-100%), followed by Daegu (-13.93%), whereas Chungcheongnam was the local administrative district with the largest increase in N_2O emission from manure management of dairy cattle by approximately 114.9% in 2014 compared to that in 2005. Total N_2O emission from manure management of Non-Korean native cattle between 2005 and 2014 decreased by approximately 49.10%. Local administrative district with the highest emission in 2014 was Gyeonggi (11,033 kt CO_2-eq), whereas the lowest emissions was in Daejeon (0 kt CO_2-eq). Those all local administrative districts showed a decrease in N_2O emission from non-Korean native cattle manure management. Daejeon showed the largest decrease of about 100% and Seoul with the smallest decrease of about 6.36%. Local administrative provinces that had decreases in emission above 30% during last ten years were Incheon, Gwangju, Daejeon, Ulsan, Gyeonggi, Gangwon, Chungcheongbuk, Chungcheongnam, Jeollabuk, Jeollanam, Gyeongsangbuk, Gyeongsangnam, and Jeju, with decreases rate of approximately 83.79, 92.27, 100,00, 79.99, 32.02, 46.18, 57.52, 37.63, 67.67, 44.19, 51.14, 78.20, and 78.56%, respectively. Local administrative provinces with decrease rate below 30% were Seoul (6.36%), Busan (6.86%) and Daegu (25.39%).

In contrast, total N_2O emission from manure management of Hanwoo cattle increased. Compared to emission in 2005, N_2O emissions from Hanwoo cattle manure management in 2014 increased by approximately 72.2%, although the emission decreased from 2012 by approximately (7.2%). Those 16 local administrative provinces had increased emission from manure management of Hanwoo cattle. Local administrative province with the highest N_2O emission in 2014 was Gyeongsangbuk (0.11 Mt CO_2-eq).

CO_2 emissions from direct on-farm energy use for cattle production

Direct on-farm energy use in cattle production is the use of energy for the milking, ventilation, heating and lighting, heating of water, and watering and feeding of animals. Three years of CO2 emissions from the direct on-farm energy used for cattle production in South Korea between 2005 and 2014 are shown in Table 8. The CO2 emissions from direct on-farm energy use for dairy cattle in South Korea decreased from 62,050.02 t CO2 in 2005 to 51,272.13 t CO2 in 2011, with an increase to 55,471.87 t CO2 in 2014. The local province of Chungcheongnam was the only province to show an increase in CO2 emissions from direct on-farm energy used in dairy cattle production of approximately 568.31 t CO2 for the ten-year period. In the other 15 local administrative provinces, the CO2 emissions from direct on-farm energy use for dairy cattle production in South Korea decreased. The highest emissions of CO2 for dairy cattle production were in Gyeonggi (21,580.82 t CO2) in 2014; however, these emissions were decreased compared to those in 2005 by approximately 23,969.34 t CO2. The decrease in percent CO2 emissions from the direct on-farm energy use in dairy cattle production between 2005 and 2014 ranged from 6.01% (Busan) to 100% (Daejeon), with an average decrease of approximately 23.20%.

Table 8. CO_2 emissions from the direct on-farm energy use for cattle production in South Korea (2005-2014)

Year	Korean native (Hanwoo) cattle (Mt CO_2-eq/year)	Non-Korean native cattle (Mt CO_2-eq/year)	Dairy cattle (Mt CO_2-eq/year)	Total (Mt CO2-eq/year)
2005	0.1490	0.0172	0.0621	0.2283
2006	0.1690	0.0172	0.0598	0.2461
2007	0.1901	0.0165	0.0579	0.2646
2008	0.2166	0.0159	0.0566	0.2891
2009	0.2377	0.0149	0.0561	0.3087
2010	0.2677	0.0154	0.0553	0.3383
2011	0.2822	0.0144	0.0513	0.3479
2012	0.2912	0.0124	0.0533	0.3570
2013	0.2900	0.0116	0.0546	0.3561
2014	0.2771	0.0095	0.0555	0.3421

The emissions from direct on-farm energy use of Non-Korean native cattle production in South Korea decreased by about 0.44% during last ten years. All the 16 local administrative provinces showed decreases in the emissions from direct on-farm energy use for Non-Korean native cattle production. The local administrative district showed the highest emissions from direct on-farm energy use in Non-Korean native cattle production in 2014 was Gyeonggi (3,559.52 kt CO_2-eq), and the lowest emissions was in Daejeon (0 t CO_2-eq) since Non-Korean native cattle population has not been growth in Daejeon on 2012. CO_2 emission decreased by more than 50% during the last ten-year period in 7 local administrative provinces, which were Incheon (-81.94%), Gwangju (-91.87%), Daejeon (-100%), Ulsan (-80.52%), Chungcheongbuk (-51.96%) Jeollabuk (-63.28%), Gyeongsangnam (-75.11%), and Jeju (-79.89%). The decreases in CO_2 emission were less than 50% in 8 local administrative provinces, which were Seoul (-15.16%), Busan (-23.80%), Daegu (-10.94%), Gyeonggi (-25.55%), Gangwon (-47.45%), Chungcheongnam (-28.85%), Jeollanam (-43.11%), and Gyeongsangbuk (-44.75%). Decrease in CO_2 emission from direct on-farm energy use in Non-Korean native cattle production was likely because of the decrease in the population of Non-Korean native cattle in those areas. Average decrease in CO_2 emission from direct on-farm energy use in Non-Korean native cattle production between 2005 and 2014 in South Korea was approximately 54.01%.

In contrast to that of Non-Korean native cattle production, CO_2 emission from the on-farm energy use in Hanwoo cattle production increased from 2005 to 2012, then thereafter start to decrease. CO_2 emission from direct on-farm energy use in the production of Hanwoo cattle in 2014 increased by approximately 85% compared to that in 2005. Local administrative district with the highest CO_2 emission from direct on-farm energy use in the production of Hanwoo cattle in 2014 was Gyeongsangbuk (59,820.40 kt CO_2-eq). Seoul was the district with the lowest CO_2 emission of approximately 8.23 kt CO_2-eq. Four local administrative provinces that increase in CO_2 emission from direct on-farm energy use in Hanwoo cattle production above 100% during the last ten years were Incheon (283.89%), Gyeonggi (127.74%), Jeollabuk (122.92%) and Jeju (102.78%). Development of Hanwoo cattle industry has been focused in these areas during last ten years, and therefore, the energy used for operating farm facilities increased as the increase of Hanwoo cattle population.

Global warming potential of GHG emission from cattle production

Global warming potential of the GHG emission from cattle production in South Korea between 2005 and 2014 is shown in Figure 1. Total global warming potential of the GHG emission from cattle production in South Korea was approximately 60.7 Mt CO2-eq between 2005 and 2014. The GWPs of emission increased from 4.67 Mt CO2-eq in 2005 to the highest emission in 2012 of about 6.97 kt CO2-eq, which then decreased to 6.65 Mt CO2-eq in 2014. The largest contribution to emission from cattle production was methane from enteric fermentation, which was approximately 50.72 Mt CO2-eq and comprised 83.52% of the total emission. The contribution from N_2O from manure management was less (6.173 Mt CO2-eq, 10.16% of total emissions), from CO_2 from direct on-farm energy use was 3.078 Mt CO2-eq (5.1% of total

emissions) and methane from manure management was 0.754 Mt CO_2-eq (1.2% of total emissions).

During the ten-year period (2005-2014), total emission from enteric fermentation from production of cattle in South Korea increased by approximately 42%. For comparison, GIR (2013) reported that total emission from enteric fermentation in a 10-year period in South Korea increased by approximately 9.7%. The estimation was lower than that of this study. High increase in emission from enteric fermentation of cattle in the ten years of this study might represent the increase in the contribution of cattle production to total methane emission. The differences between the two estimates were primarily because the IPCC Tier-2 model significantly overpredicts cattle GE intake at higher level intake. This over predict was also reported by Jo et al. (2015) with Hanwoo steers study. Furthermore, it is consensused that the enteric

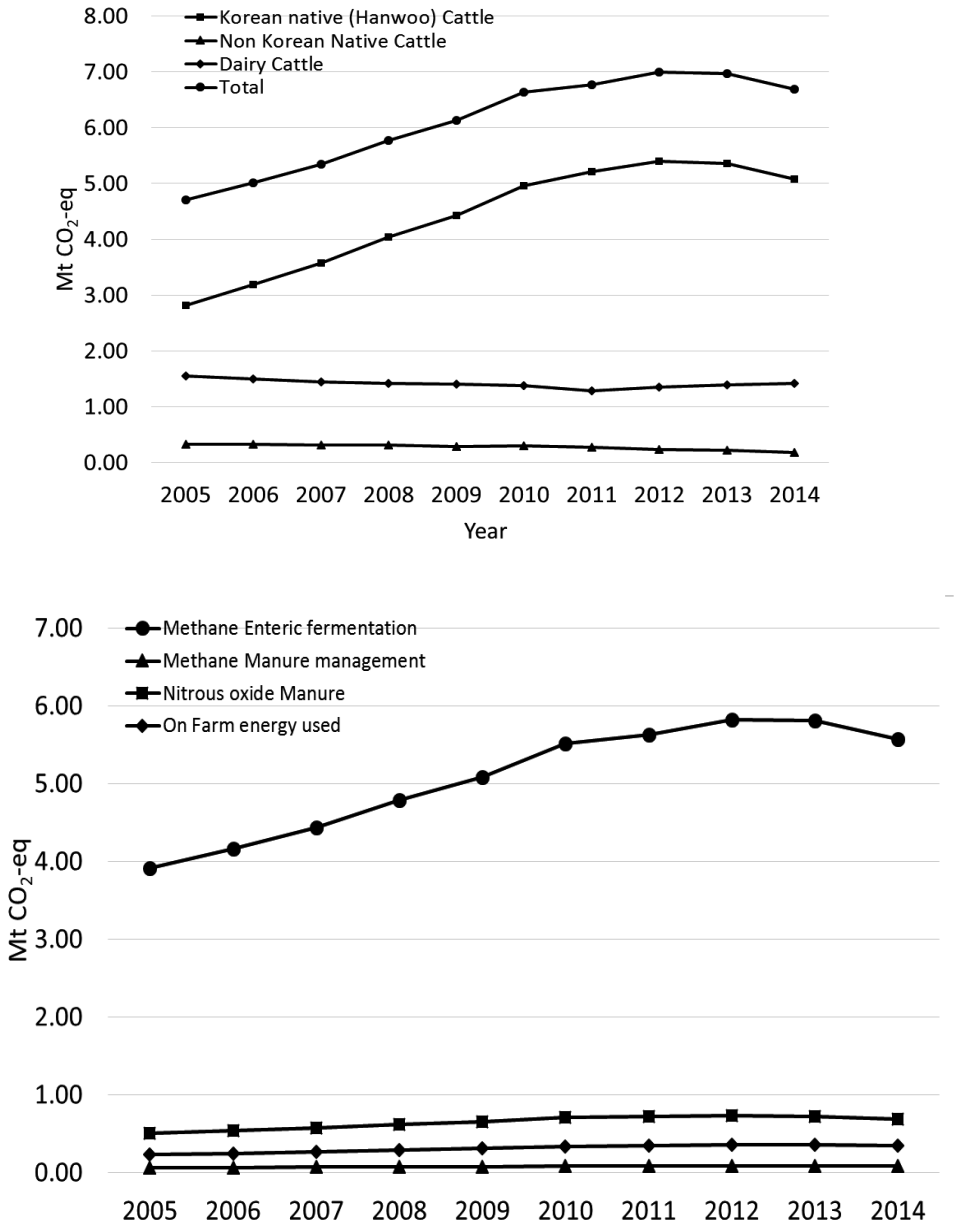


Figure 1. Global warming potential of GHG emission by cattle type (A) and by emission type (B) from cattle livestock production activities in South Korea in between 2005-2014.

fermentation of cattle is the largest source of CH_4 emission in the livestock sector (FAO 2006).

The highest emission of greenhouse gases during the ten-year period (2005-2014) of approximately 44.081 Mt CO2-eq was generated in the production of Hanwoo cattle, which was followed by dairy cattle production (13.84 Mt CO2-eq) and Non-Korean native production (2.80 kt CO2-eq). Compared with the level in 2005, the greenhouse gas emission from production of Hanwoo cattle have not reached the declared target level of a 30% reduction by 2020 (4% lower than the 2005 level) with 5.08 Mt CO2-eq produced in 2014, which was 80% higher than that in 2005 (2.82 Mt CO2-eq). However, target levels for the reduction in greenhouse gas emission from the production of both Non-Korean native beef and dairy cattle were reached. Emissions from production of Non-Korean native cattle were 0.18 Mt CO2-eq in 2014, which was 45% lower than that in 2005 (0.33 Mt CO2-eq).

Annual average and annual growth rate of GHG emission from cattle production in South Korea between 2005 and 2014 are shown in Figure 2. Annual average GHG emission from Hanwoo cattle production was 4,408.13 kt CO2-eq/y, which was higher than that Non-Korean native cattle production (280.46 kt CO2-eq/y) and dairy cattle production (1,384.17 kt CO2-from production of Hanwoo cattle was 6.06% between 2005 and 2014. By contrast, average annual growth rate of GHG emission from beef and dairy cattle production was -5.83% and -0.92%, respectively (eq/y). Average annual growth rate of GHG emission

Annual average GHG emission from the production of Hanwoo beef and dairy cattle by province in South Korea between 2005 and 2014 are shown in Figure 3. Local administrative district with the highest annual average GHG emission from the production of Hanwoo cattle was Gyeongsangbuk (874,088.35 t CO2-eq/y), whereas Seoul had the lowest emissions (309.28 t CO2-eq/y). In 8 local administrative provinces, annual average GHG emission from the production of Hanwoo cattle was above 200,000 t CO2-eq/y, which included Gyeonggi (329,236.88 t CO2-eq/y), Gangwon (333,708.28 t CO2-eq/y), Chungcheongbuk (276,040.47 t CO2-eq/y), Chungcheongnam (545,616.62 t CO2-eq/y), Jeollabuk (483,579.63 t CO2-eq/y), Jeollanam (717,474.71 t CO2-eq/y), Gyeongsangbuk (874,088.35 t CO2-eq/y), and Gyeongsangnam (459,675.92 t CO2-eq/y). The highest annual growth rate in GHG emissions from the production of Hanwoo cattle was in Incheon (12.73% per year), and the lowest annual growth rate was in Seoul (1.99% per year). In contrast to the emission from production of Hanwoo cattle, the highest annual average of GHG emission from beef and dairy cattle production was in Gyeonggi Province, with 102,811.80 and 579,239.60 t CO_2-eq/y, respectively. Daejeon was the local administrative province with the lowest annual growth rates for the emissions from beef and dairy cattle production, with approximately -27.72% and -47.81% per year, respectively. From this study, the local towns primarily produced the GHG emission from the production of Hanwoo cattle compared with the metropolitan areas. The annual growth rate of GHG emissions from production of Hanwoo cattle was considerably higher than that of beef and dairy cattle production.

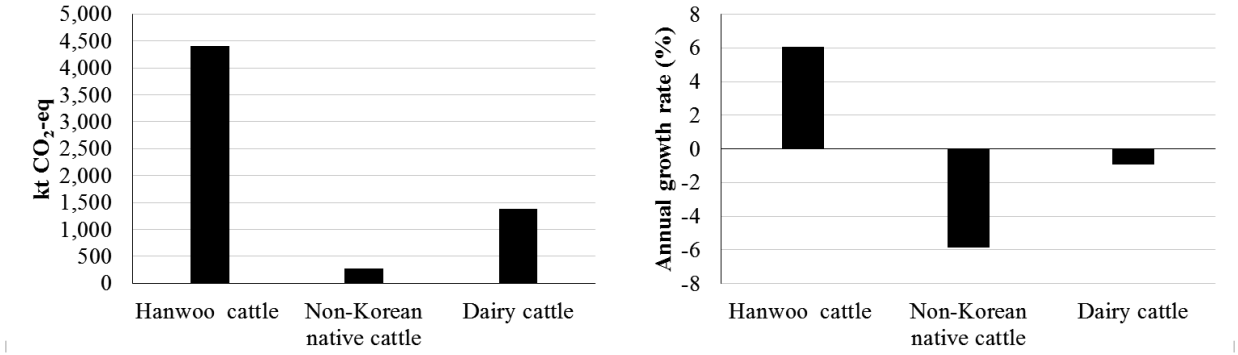


Figure 2. Annual average (A) and annual growth rate (B) of GHG emission from cattle production in South Korea (2005-2014).

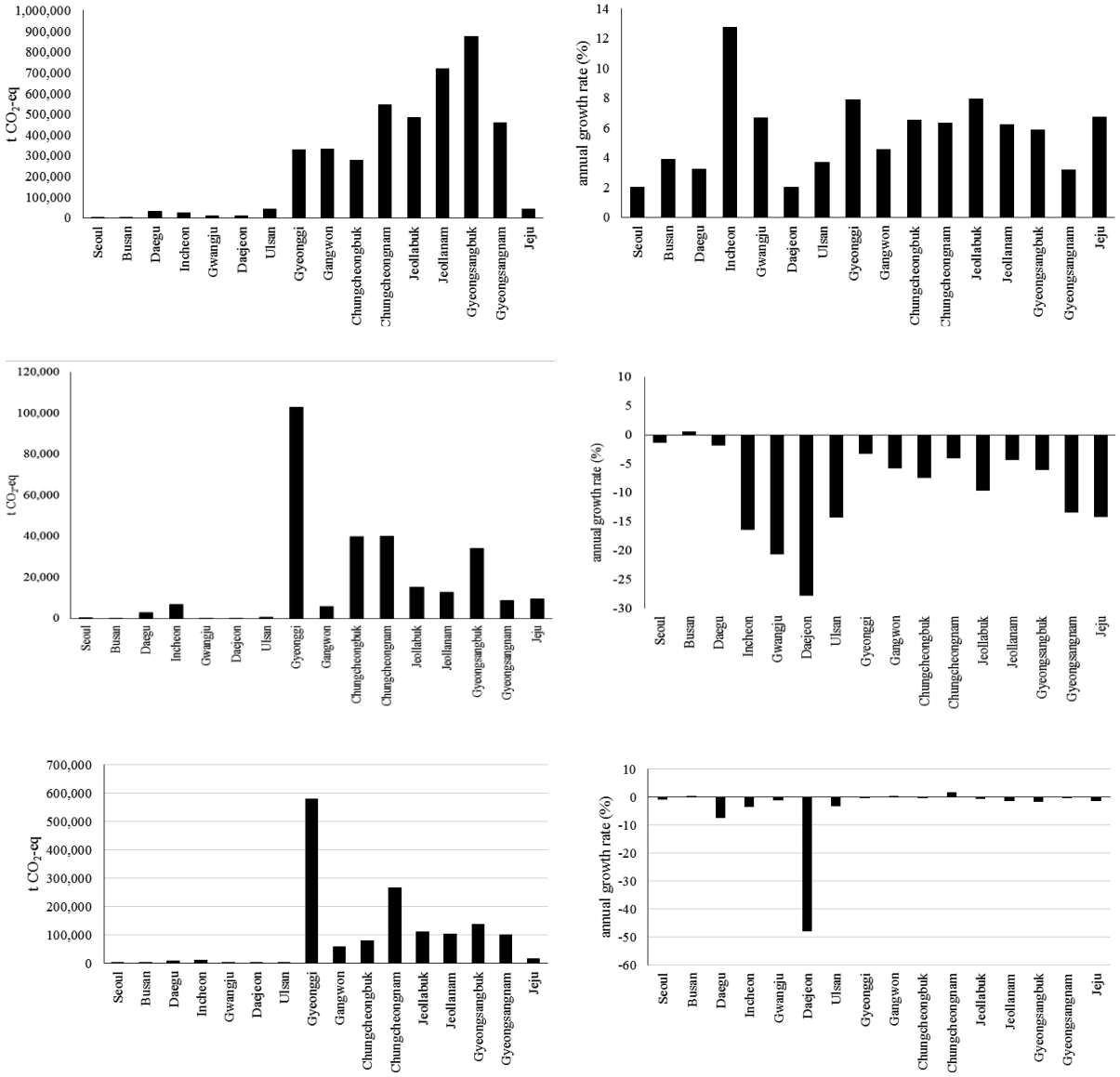


Figure 3. Annual average and annual growth rate of GHG emission from the production of Hanwoo cattle (A), Non-Korean native cattle (B) and Dairy cattle (C) by province in South Korea (2005-2014).

Results of correlation analyses between the cattle type and GHG emission in South Korea from 2005 to 2014 are provided in Table 9. Correlation coefficient of CH_4 emission from the enteric fermentation of Hanwoo cattle and the total CH_4 emission was 0.999, which was higher than that Non-Korean native cattle (-0.823) or dairy cattle (-0.947). Correlation coefficient of CH_4 emission from the manure management of Hanwoo cattle and total CH_4 emission was 0.998, which was higher than that Non-Korean native cattle (-0.817) or dairy cattle (-0.944). Correlation coefficient of NO_2 emission from manure management of Hanwoo cattle and total NO_2 emission was 0.956, which was higher than that Non-Korean native cattle (-0.715) or dairy cattle (-0.934). Correlation coefficient of CO_2 emission from on-farm energy use in production of Hanwoo cattle and total CO_2 emission was 0.999, which was higher than that Non-Korean native cattle (-0.819) or dairy cattle (-0.951). Correlation coefficient was 0.999 between the global warming potential of GHG emission from production of Hanwoo cattle and total global warming potential of GHG emission, which was higher than that Non-Korean native cattle (-0.818) or dairy cattle (-0.949). Those showed that production of Hanwoo cattle was the primary contributor to total CH4 emission from enteric fermentation, CH4 emission from

Table 9. Correlation analysis between cattle type and GHG emission in South Korea from 2005 to 2014

	CH4 enteric fermentation	CH4 manure management	NO2 manure management	CO2 on farm energy use	Global warming potential
Hanwoo	0.999	0.998	0.956	0.999	0.999
Non-Korean native cattle	-0.823	-0.817	-0.715	-0.819	-0.818
Dairy	-0.947	-0.944	-0.934	-0.951	-0.949

manure management, NO2 emission from manure management, CO2 emission from on-farm energy use, and total global warming potential of GHG emission from cattle production sector in South Korea.

However, both overestimates and underestimates of emissions were possible in this study. Kebreab et al. (2008) reported that the IPCC values result in an overestimate of emissions by approximately 12.5% and an underestimate by approximately 9.8% for dairy and feedlot cattle, respectively. In this study, about 31.18% overestimates of enteric fermentation occurred which was higher than the one reported by GIR (2013).

CONCLUSION

Emissions of greenhouse gases from the cattle production sector in South Korea increased year to year and failed to reach the target pledge of a 30% reduction compared with current levels. Production of Hanwoo cattle was the primary contributor to CH_4 emission from enteric fermentation, CH_4 emission from manure management, NO_2 emission from manure management and CO_2 emissions from on farm energy use in the cattle production sector of South Korea during the ten-year period of this study. Proper mitigation is required in the cattle production sector to meet the target pledge for emission in 2020. Mitigation options for reductionsin emission should focus on the production of Hanwoo cattle because of the significant contributions to greenhouse gas emission in South Korea.

ACKNOWLEDGEMENTS

This work was supported by the Animal Environmental and Bioengineering Laboratory, Department of Agricultural Biotechnology, Seoul National University (SNU), Seoul, South Korea. The authors would like to thank the Korean Institute of Planning & Evaluation for Technology in Food, Agriculture, Forestry & Fisheries (IPET) and the Korean Energy Technology Evaluation and Planning (KETEP) for financial support during this study.

REFERENCES

[AAFCCT] Agriculture and Agri-Food Climate Change Table. 2000. Options report: Reducing greenhouse gas emissions from Canadian Agriculture. Publication No. 2028/E.

Amon B, Kryvoruchko V, Amon T, Zechmeister-Boltenstern S. 2006. Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. Agric Ecosyst Environ. 112:153-162. doi: 10.1016/j.agee.2005.08.030.

Basarab JA, Okine EK, Baron VS, Marx T, Ramsey P, Ziegler K, Lyle K. 2005. Methane emissions from enteric fermentation in Alberta's Non-Korean native cattle population. Can J Anim Sci. 501:2-13. doi: 10.4141/A04-069.

[FAO] Food and Agriculture Organization. 2006. Livestock's long shadow: Environmental issues and options. Rome (Italy): Food and Agriculture Organization of the United Nations.

Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A, Tempio G. 2013. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Rome (Italy): Food and Agriculture Organization, United Nations.

[GIR] Gas Inventory and Research. 2013. National greenhouse gas inventory report of Korea. Seoul (Korea): Greenhouse Gas Inventory and Research Center of Korea.

Höglund-Isaksson L. 2012. Global anthropogenic methane emissions 2005-2030: Technical mitigation potentials and costs. Atmosph Chemist Phys. 12:9079-9096. doi: 10.5194/acp-12-9079-2012.

[IPCC] Intergovernmental Panel on Climate Change. 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme. Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K, editors. Kanalawa (Jpn): Institute for Global Environmental Strategies.

Ji ES, Park KH. 2012. Methane and nitrous oxide emissions from livestock agriculture in 16 local administrative provinces of Korea. Asian-Aust J Anim Sci. 25:1768-1774. doi: 10.5713/ajas.2012.12418.

Jo N, Kim J, Seo S. 2015. Estimation of methane emission factor for enteric fermentation of growing-finishing Hanwoo steers using the IPCC Tier 2 approach. Peer J Pre Prints 3:e1578. doi: 10.7287/peerj.preprints. 1285v1.

Johnson KA, Johnson DE. 1995. Methane emissions from cattle. J Anim Sci. 73:2483-2492. doi: 10.2527/1995. 7382483x.

Kebreab E, Johnson KA, Archibeque SL, Pape D, Wirth T. 2008. Model for estimating enteric methane emissions from United States dairy and feedlot cattle. J Anim Sci. 86:2738-2748. doi: 10.2527/jas.2008-0960.

Lee HJ, Lee SC. 2003. National methane inventory relevant to livestock enteric fermentation. Korean J Anim Sci Technol. 45:997-1006. doi: 10.5187/JAST.2003. 45.6.997.

Merino P, Ramirez-Fanlo E, Arriaga H, del Hierro O, Artetxe A, Viguria M. 2011. Regional inventory of methane and nitrous oxide emission from ruminant livestock in the Basque country. Anim Feed Sci Technol. 166-167:628-640.

Opio C, Gerber P, Mottet A, Falcucci A, Tempio G, MacLeod M, Vellinga T, Henderson B, Steinfeld H. 2013. Greenhouse gas emissions from ruminant supply chains-A global life cycle assessment. Rome (Italy): Food and Agriculture Organization, United Nations.

Patra AK. 2014. Trends and projected estimates of GHG emissions from Indian livestock in comparisons with GHG emissions from world and developing countries. Asian Australas J Anim Sci. 27:592-599. doi: 10.5713/ajas.2013.13342.

Smith P, Martino D, Cai Z, Gwary D, Janzen H, Kumar P, McCarl B, Ogle S, O'Mara F, Rice C, Scholes B, Sirotenko O. 2007. Agriculture. In: Metz, B, Davidson OR, Bosch PR, Dave R, Meyer LA, editors. Climate change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge (UK): Cambridge University Press.

[UNFCCC] United Nations Framework Convention on Climate Change. 2011. FCCC/AWGLCA/2011/INF.1. United Nations.

[USEPA] United States of Environmental Protection Agency. 2014. Climate change indicators in the United States, 2014. 3th ed. EPA 430-R-14-004. www.epa.gov/ climatechange/indicators.

Effect of Vitamin C Administration in Diluent Media to Quality of Dairy Cattle Thawed Spermatozoa

Afiati F[1], Lestari DA[2], Malini DM[2]

[1]*Biotechnology Research Center-IIS, Jl. Raya Bogor Km. 46 Cibinong 16911*
[2]*Study Program of Biology, Faculty of Mathemathic and Natural Science, Padjajaran University*
Jl. Raya Bandung-Sumedang Km. 21 Jatinangor 45363
E-mail: afiati.biotek@gmail.com

(received 10-03-2016; revised 31-05-2016; accepted 13-06-2016)

ABSTRAK

Afiati F, Lestari DA, Malini DM. 2016. Pengaruh penambahan vitamin C dalam media pengencer terhadap kualitas spermatozoa sapi perah setelah *thawing*. JITV 21(2): 124-134. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1360

Proses pembekuan dan *thawing* semen, dapat menyebabkan kematian spermatozoa dan spermatozoa yang bertahan hidup mempunyai fertilitas yang rendah. Tujuan dari penelitian ini untuk mendapatkan konsentrasi vitamin C yang optimum dalam media pengencer untuk meningkatkan kualitas spermatozoa sapi perah (*Bos taurus*) tanpa *sexing* dan hasil *sexing* setelah *thawing*. Metode yang digunakan adalah metode eksperimental dengan rancangan acak lengkap pola faktorial 3x4. Rancangan ini terdiri dari dua faktor yaitu pemisahan spermatozoa dan konsentrasi vitamin C. Faktor pemisahan spermatozoa yaitu spermatozoa tanpa *sexing* dan hasil *sexing* (X dan Y). Faktor konsentrasi vitamin C yaitu 0% (K), 0,25% (P_1), 0,50% (P_2) dan 0,75% (P_3). Data dianalisis menggunakan analisis variansi (ANAVA) dan uji Jarak Berganda Duncan 95%. Konsentrasi vitamin C yang optimum dalam media pengencer untuk meningkatkan motilitas spermatozoa sapi perah (*Bos taurus*) tanpa sexing setelah thawing adalah 0,25% dan 0,5%. Sedangkan untuk meningkatkan motilitas spermatozoa sapi perah (*Bos taurus*) hasil sexing, baik spermatozoa X maupun spermatozoa Y setelah thawing adalah 0,50%. Viabilitas spermatozoa tanpa sexing dengan penambahan vitamin C 0,75% pada media pengencer lebih rendah dibanding dengan semen dalam pengencer tanpa penambahan viamin C. Penambahan vitamin C pada pengencer tidak mempengaruhi tingkat abnormalitas spermatozoa tanpa sexing dan spermatozoa X, tapi penambahan vitamin C pada pengencer 0,25% tidak berbeda dengan penambahan vitamin C 0,5%. Penambahan vitamin C dalam media pengencer dapat mempengaruhi tingkat motilitas, viabilitas, abnormalitas dan integritas membran plasmaa spermatozoa tanpa *sexing* dan hasil *sexing* pada sapi perah (*Bos taurus*) setelah *thawing*.

Kata Kunci: Spermatozoa, Vitamin C, *Sexing*, Pengencer, *Thawing*

ABSTRACT

Afiati F, Lestari DA, Malini DM. 2016. Effect of addition of vitamin C in diluent medium for quality of dairy cow spermatozoa after thawing. JITV 21(2): 124-134. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1360

The process of freezing and thawing of semen could lead spermatozoa death and low fertility for alive spermatozoa. This research was subjected to determine the optimum concentration of vitamin C in diluent media to improve the quality of non-sexed and sexed of thawed dairy cattle (*Bos taurus*) spermatozoa. The method used was completely randomized design with 3x4 factorial consisting of spermatozoa isolation and vitamin C concentration. Spermatozoa isolation factors were non-sexed and sexed (X and Y) spermatozoa. Vitamin C concentrations factors were 0% (K); 0.25% (P_1); 0.50% (P_2) and 0.75% (P_3). Data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test 95%. The optimum concentration of vitamin C in diluent media to improve the quality of non-sexed thawed dairy cattle (*Bos taurus*) spermatozoa was 0.25%. While the optimum concentration of vitamin C in diluent medium to improve quality of thawed dairy cattle (*Bos taurus*) spermatozoa both X and Y was 0.50%. Administration of vitamin C in diluent media could improve the quality of thawed non-sexed and sexed (X and Y spermatozoa) spermatozoa in dairy cattle (*Bos taurus*).

Kata Kunci: Spermatozoa, Vitamin C, Sexing, Diluent, Thawing

INTRODUCTION

Dairy milk yield has not fulfilled national milk requirement due to the small population of dairy cattle. National dairy cattle population is about 525,171 heads (CSA 2015). It only increases by 4.31% (502,516 heads) from 2014. The National requirement of fresh milk is 3.3 million ton/year, otherwise, its availability only supplies about 690 hundred ton/year (21%) and the rest are fulfilled by imported 2.61 million ton/year (79%) (Ministry of Industry 2013). Dairy cattle population could be increased by Artificial Insemination (AI) and use of sexing process to increase desired sex of cattle.

Currently, the most AI used frozen semen. Frozen semen may be used for long term. However, freezing process leads to spermatozoa death up to 50% and low fertility for the alive one (Lessard et al. 2000). Thawing is a re-melting process using certain media and duration before the spermatozoa used. This thawing process generated heat shock effect or contamination of oxygen to spermatozoa influencing plasma membrane stability and then it influenced the quality of spermatozoa (Salim et al. 2012).

The sexing process also induced damage to the plasma membrane, mitochondria veil, releasing of various enzymes, the decrease of lipoprotein and amino acid level, agglutination of spermatozoa head leading to decrease of motility and fertility of spermatozoa, even the spermatozoa death.

Winarto (2010) said that spermatozoa damage by oxidative stresses developed by free radicals against normal cells, protein, and fat. Yuliani & Lukman (2013) also said that the alive spermatozoa were very sensitive to the external environment. Temperature change and extreme osmolarity during AI process might lead to Reactive Oxygen Species (ROS) production (Nebel 2007; Moore et al. 2005; Sukmawati et al. 2014). The quality of sperm might be improved after freezing process by administration of Vitamin C in diluent media. Vitamin C was an antioxidant leading to decrease the sensitivity of plasma membrane of spermatozoa against lipid peroxidation due to ROS process (Sugiarti et al. 2001).

The quality of thawed sperm administered by 0.02% vitamin C into diluent media did not different in motility, abnormality and viability with the control, but showed significant different ($P<0.05$) in the integrity of plasma membrane (Afiati et al. 2014). Franco et al. (2013) reported that supplementation of ascorbate acid in semen diluent did not show a positive effect in 0.45 g/L but positive in 0.9 and 1.8 g/L concentration against integrity and stability of plasma membrane.

Study of antioxidant administration has been widely provided, however, the study of the administration of vitamin C into diluent media to determine the quality of spermatozoa of dairy cattle (*Bos taurus*) without and with good sexing after thawing has not been widely done. Therefore, the study of the effect of vitamin C administration into diluent media against the quality of dairy cattle of non-sexed and sexed spermatozoa after thawing is needed to be done. This study aim was to obtain optimum vitamin C concentration in diluent media to improve the quality of dairy cattle (*Bos taurus*) of thawed non-sexed and sexed spermatozoa.

MATERIALS AND METHODS

This research was conducted in Laboratory of Breeding Reproduction and Animal Cells Culture of Indonesian Research Institute for Biotechnology and Bioindustry, Bogor from April – July 2015. Semen samples were from one 4-years old Friesian Holstein. Semen was collected using artificial vagina equipped by scale container glass tube. Collected semen was immediately observed macroscopically (for color, odor, volume, pH, and its consistency) and microscopically (for its concentration, mass movement, motility, viability, abnormality, the integrity of whole plasma membrane and nucleus integrity). Good semen categories were having minimal mass movement (thin, infrequent, and slow movement), motility ≥70%, abnormality <20%, and concentration >1000 x 106 ml (Arifiantini 2012).

Spermatozoa sexing process

Good semen and spermatozoa were sexed using multilevel albumin method using Bovine Serum Albumin (BSA 5% for the upper column and 10% for under column). Then 1 ml semen was slowly added and aged for 60 minutes on that upper multilevel albumin column. Upper albumin column (BSA 5%) was predicted as spermatozoa X and the under albumin column (BSA 10%) as spermatozoa Y (Hendri 1992; Kaiin et al. 2003).

Dilution, packaging, equilibration, freezing and thawing

Non-sexed and sexed spermatozoa were then diluted using Tris-Stirat buffer (Table 1). Interval time between semen collections to the dilution was no more than 15 minutes. Administration of vitamin C in diluent media was adapted with treatments (0, 0.25, 0.5, and 0.75% concentration).

Table 1. Composition of tris-citric buffer diluent

Material	Amount
Tris (Hydroxymethyl aminomethane) (g)	3.028
Citric acid (g)	1.675
Fructose (g)	1.25
Penicillin (g)	0.0525
Streptomycin (g)	0.075
Aquabides (ml)	100
Yolk (ml)	20.0
Glycerol (ml)	6.00

(Anggraeny et al. 2004)

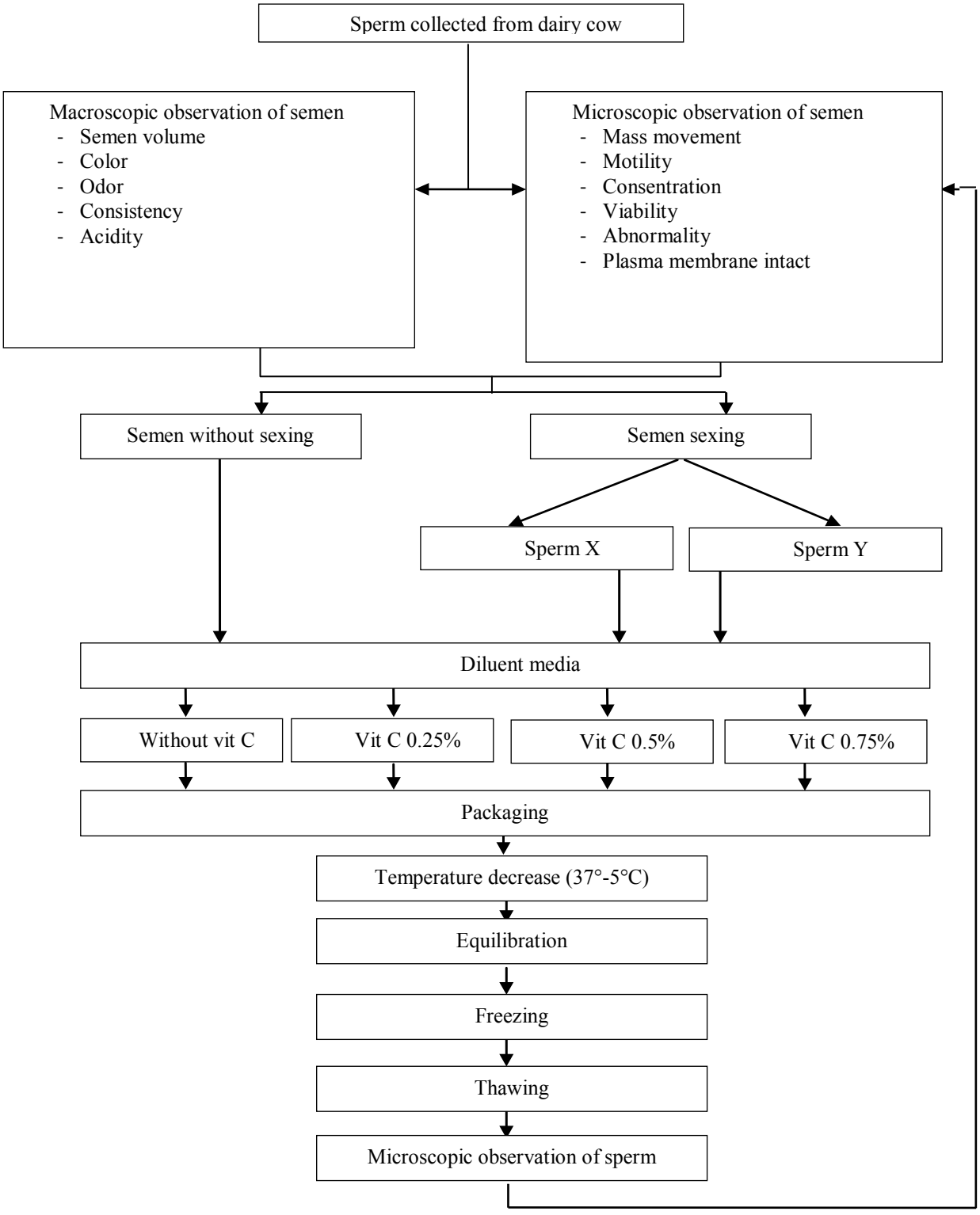


Figure 1. Flow diagram of assessment process (modified Kaiin et al. 2003).

In packaging process, 0.5 ml straw was labeled using printing tool. Semen was put in that straw using filling tool. Then the straw was sealed using sealing tool. Semen was equilibrated by placing it in 5°C temperature for 4 hours before freezing process. Sealed straws were organized in the cooling rack and then were equilibrated. Freezing process was conducted in nitrogen steam by putting sealed straw rack 10 cm

above liquid nitrogen for 15 minutes (about -130°C) and then put in -196°C liquid nitrogen for 10 minutes (Sugiarti et al. 2001). Frozen semen was thawed by putting those frozen straws into 37°C water for 30 seconds (Pratiwi et al. 2011). Observation of spermatozoa quality was conducted microscopically.

Assessment and observation of microscopic parameters

Microscopic parameters observed after thawing were the mass movement, motility, viability, abnormality, the integrity of plasma membrane and spermatozoa nucleus. This study process is showed in Figure 1.

Data analysis

This study was designed by two factors that were spermatozoa isolation and vitamin C concentration. Spermatozoa isolation factors were non-sexed and sexed (X and Y). Vitamin C factors were 0% (K), 0.25% (P1), 0.50% (P2) and 0.75% (P3). The method used in this study was an experimental method with completely randomized design 3x4 factorial pattern and 3 repetitions. Data obtained were then analyzed by 95% ANOVA (Steel & Torrie 1995).

RESULTS AND DISCUSSION

Assessment of fresh semen (Table 2) showed good result in accordance with the good quality requirement of spermatozoa, so then was continued by isolation process. The quality of sexed spermatozoa showed good score and was feasible to be frozen (Table 3). Spermatozoa quality of fresh FH semen (Table 2) used was categorized as normal spermatozoa according to Hafez & Hafez (2000): had normal ejaculation volume by 5-8 ml. Normal semen odor was similar with chlorine or acacia flower odor (Subrata 1999). Garner & Hafez (2000) said that normal ejaculation color of cattle was beige to white milk, while spermatozoa with low concentration would appear clearly and transparent. That color change was matched with pH meter. Hafez & Hafez (2000) reported that pH of fresh cattle semen was around 6.4-7.8. Those normal-categorized spermatozoa were qualified for further process.

Table 2. Quality of fresh dairy cattle semen

Parameter	Result (Average) (±SD)
Macroscopic observation	
Color	Beige – White-milk
Odor	Specific
Volume (ml)	10.5±2.18
pH	7
Consistency	aqueous – dense
Microscopic Observation	
Consentration (10^6/ml)	1440.67±699.84
Mass movement	Good
Motility (%)	79.63±7.47
Viability (%)	87.19±6.63
Abnormality (%)	6.01±1.46
Integrity of plasma membrane (%)	84.92±5.25
Integrity of nucleus (%)	96.88±5.40

Spermatozoa X and Y had qualified score for artificial insemination (Table 3). Obtained result was in accordance with research result of Afiati (2004) and Pratiwi et al. (2006) showing that percentage of motility, viability and integrity of plasma membrane, and abnormality of sexing result spermatozoa showed a qualified score for freezing.

Table 3. Quality of sexing result spermatozoa

Parameter	Spermatozoa X	Spermatozoa Y
Concentration (10^6/ml)	1022±625.63	1280±890.31
Motility (%)	82.13±6.52	85.72±4.74
Viability (%)	70.57±7.36	77.35±8.96
Abnormality (%)	5.07±1.06	4.69±1.82
Integrity of plasma membrane (%)	82.29±7.86	83.49±8.98
Integrity of nucleus (%)	99.69±0.54	99.89±0.18

Table 4. Motility of thawed dairy cattle spermatozoa

Concentration of vitamin C	Spermatozoa type (± SD) (%)		
	Non-sexed	Spermatozoa X	Spermatozoa Y
0% (control)	43.44±3.03$^{(A)a}$	48.88±6.23$^{(A)a}$	43.29±4.99$^{(A)a}$
0.25% (P1)	52.01±3.82$^{(B)a}$	41.81±3.65$^{(A)b}$	43.37±1.76$^{(A)b}$
0.50% (P2)	46.21±5.41$^{(AB)a}$	58.74±2.19$^{(B)b}$	59.99±1.47$^{(B)b}$
0.75% (P3)	42.82±2.05$^{(A)a}$	46.35±5.98$^{(A)a}$	38.24±4.28$^{(A)a}$

Different capital words in one column show significant difference (P<0.05). Different lowercase in one row shows significant different (P<0.05)

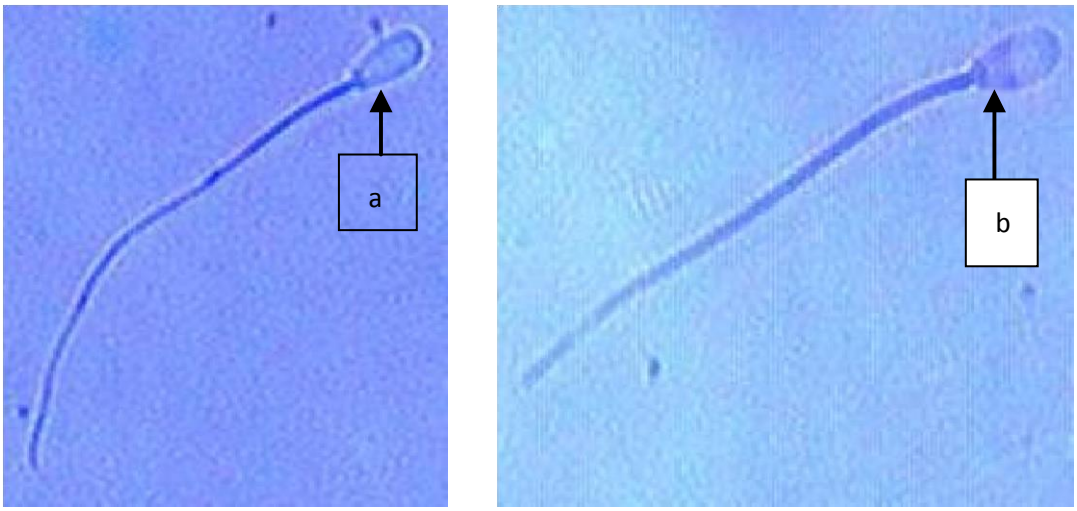


Figure 2. Viability of dairy cattle spermatozoa, (a) alive spermatozoa, (b) dead spermatozoa.

Quality of spermatozoa after thawing

Motility of thawed spermatozoa (Table 4) without vitamin C (0%) administration was significantly lower than X and Y spermatozoa. However, motility of non-sexing spermatozoa without vitamin C administration (0%) was not significantly different with the 0.5% and 0.75%, but lower than 0.25% vitamin C administration.

Motility of non-sexing spermatozoa with 0.25% vitamin C administration was significantly lower than X and Y spermatozoa. Administration of 0.25% vitamin C in non-sexing spermatozoa showed the same motility with spermatozoa in 0.5%, but was significantly higher than spermatozoa in 0% and 0.75% vitamin C administration.

Motility of non-sexing spermatozoa with 0.5% vitamin C administration was significantly lower than Spermatozoa X and Y. Administration of 0.5% vitamin C in non-sexing spermatozoa had no different motility with that at 0.25%, but was significantly different with the 0% or 0.75% vitamin C administrations.

Motility of non-sexing spermatozoa with 0.75% vitamin C administration did not show significant different than Spermatozoa X or Y. Administration of 0.75% vitamin C in non-sexing spermatozoa was not different with that 0% or 0.5%, but significantly lower than the 0.5% vitamin C administration.

Motility percentage of spermatozoa (Table 4) observed was in accordance with Savitri et al. (2014) and Aslam et al. (2014). Savitri et al. (2014) reported that motility percentage of Bali cattle thawed spermatozoa was 40.00±5.00% with administration of 26.43% vitamin C in diluent media. Aslam et al. (2014) reported that administration of 0.50% vitamin C showed higher motility in Aceh cattle thawed spermatozoa than control (40.16±3.50%). Administration of vitamin C was able to improve motility of spermatozoa due to optimization of fructolysis fulfilling energy requirement in the form of ATP (Sumargono 1998). Aslam et al. (2014) said that vitamin C was able to bind radical oxygen in the cells preventing lipid peroxidation that might prevent motility. Vitamin C was a soluble vitamin which was able to protect spermatozoa from damage caused by oxidative stress by neutralizing hydroxyl, super-oxidation and peroxide hydrogen radical and prevent spermatozoa agglutination (Agarwal & Sekhon 2010; Sitohang et al. 2015).

Table 5. Viability of thawed dairy cattle spermatozoa

Concentration of vitamin C	Spermatozoa type (± SD) (%)		
	Non-Sexing	Spermatozoa X	Spermatozoa Y
0% (control)	68.31±7.28$^{(AB)}$	73.14±7.34$^{(A)}$	70.30±5.98$^{(A)}$
0.25% (P1)	75.04±7.03$^{(B)}$	73.29±9.56$^{(A)}$	74.47±7.93$^{(A)}$
0.50% (P2)	73.70±7.99$^{(B)}$	74.23±8.73$^{(A)}$	76.89±8.87$^{(A)}$
0.75% (P3)	65.49±9.74$^{(A)}$	67.72±7.48$^{(A)}$	71.37±8.09$^{(A)}$

Different capital words in one column shows significant difference (P<0.05)

Table 6. Abnormality of thawed dairy cattle spermatozoa

Concentration of Vitamin C	Spermatozoa type (± SD) (%)		
	Non-Sexing	Spermatozoa X	Spermatozoa Y
0% (control)	6.74±0.99$^{(A)}$	6.97±1.17$^{(A)}$	6.95±1.31$^{(A)}$
0.25% (P1)	5.96±1.51$^{(A)}$	6.37±1.78$^{(A)}$	6.11±1.50$^{(AB)}$
0.50% (P2)	6.60±1.64$^{(A)}$	5.92±0.93$^{(A)}$	5.36±1.61$^{(B)}$
0.75% (P3)	6.68±1.27$^{(A)}$	6.76±1.65$^{(A)}$	6.86±1.01$^{(A)}$

Different capital words in one column shows significant difference (P<0.05)

Observation of spermatozoa viability (Table 5) was conducted to eosin-nigrosin colored spermatozoa. Spermatozoa which absorbed color was dead spermatozoa, meanwhile, spermatozoa which did not absorb color (green) was alive spermatozoa (Figure 2). Putra et al. (2012) said that permeability of dead spermatozoa membrane was increase, so that eosin-nigrosin dye easily passed the membrane and entered spermatozoa. Meanwhile, the permeability of alive spermatozoa membrane remained normal, so that eosin-nigrosin dye could not pass the membrane. Thawed spermatozoa were more sensitive and easily dead. Park & Graham (1992) said that spermatozoa lost their viability during freezing process due to change of structure and membrane function.

Viability (Table 5) in non-sexing spermatozoa without vitamin C (0%) administration was not different with the 0.25% and 0.5%, but was significantly higher than the 0.75% vitamin C administration.

Administration of 0.25% vitamin C to non-sexed spermatozoa showed the same viability with 0% and 0.5%, but was significantly higher than the 0.75% vitamin C administration.

The viability of non-sexed spermatozoa administered by 0.5% vitamin C was not different with 0% and 0.25%, but was significantly higher than 0.75% vitamin C administration.

The viability of non-sexed spermatozoa administered by 0.75% vitamin C was not different with the 0%, but was significantly higher than 0.25% and 0.5% vitamin C administration.

The viability of X and Y spermatozoa did not significantly different either in the 0.25% or 0.75% vitamin C administration.

Research results showed that administration of vitamin C in diluent media might be a reference for spermatozoa viability. Viability percentage obtained in this study was in accordance with Savitri et al. (2014) reporting that viability percentage of thawed Bali cattle spermatozoa with 44.03% vitamin C administration in diluent media was 54.33±3.51%.

Administration of vitamin C in diluent media is one of the factors increasing spermatozoa viability. Vitamin C, in addition to counteracting free radicals, was also to optimize fructolysis decelerating damage of spermatozoa membrane permeability and consequently extend the life of spermatozoa (Hidayaturrahmah 2007).

Abnormality (Table 6) of non-sexing and spermatozoa X did not different in each vitamin C administration level. Abnormality in spermatozoa Y with 0% vitamin C administration did not different with the 0.25% and 0.75%, but was significantly higher than 0.5% vitamin C administration.

Abnormality observed in this study was morphological abnormalities. Abnormality of spermatozoa was caused by various factors such as: diseases, temperature, season, treatments during cryopreservation and technique of semen collection and

Table 7. Integrity of plasma membrane of thawed dairy cattle spermatozoa

Concentration of Vitamin C	Spermatozoa type (± SD) (%)		
	Non-Sexing	Spermatozoa X	Spermatozoa Y
0% (control)	37.72±4.08$^{(A)a}$	32.11±4.15$^{(A)b}$	36.03±3.35$^{(A)a}$
0.25% (P1)	38.38±5.98$^{(A)a}$	36.54±4.73$^{(AB)a}$	36.49±4.31$^{(A)a}$
0.50% (P2)	37.70±4.78$^{(A)a}$	37.44±4.18$^{(B)a}$	37.20±6.53$^{(A)a}$
0.75% (P3)	27.77±5.77$^{(B)a}$	34.69±5.57$^{(AB)b}$	34.25±3.20$^{(A)b}$

Different capital words in one column shows significant difference (P<0.05). Different lowercase in one row shows significant different (P<0.05)

Table 8. Integrity of nucleus of thawed dairy cattle spermatozoa

Concentration of vitamin C	Spermatozoa type (± SD) (%)		
	Non-*Sexing*	Spermatozoa X	Spermatozoa Y
0% (control)	99.78±0.44	99.18±0.86	99.73±0.54
0.25% (P1)	99.89±0.33	99.63±0.58	99.73±0.57
0.50% (P2)	99.79±0.41	99.78±0.44	99.90±0.29
0.75% (P3)	99.82±0.36	99.68±0.48	99.58±0.84

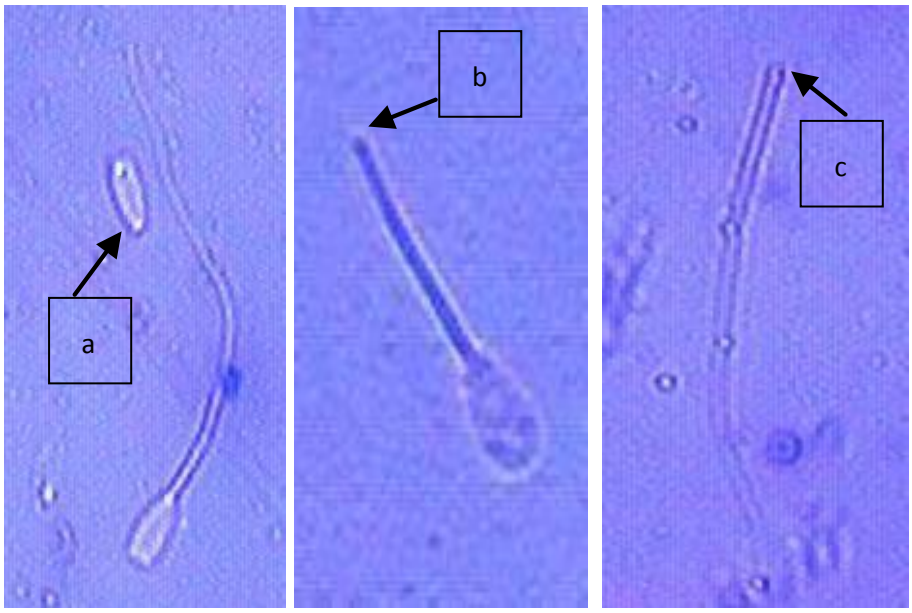


Figure 3. Abnormality of dairy cattle spermatozoa. (a) head without tail, (b) break tail, (c) tail without head.

staining (Sukmawati et al. 2014). Results of abnormality observation (Table 6) showed that abnormality commonly found was secondary abnormality such as separated head-tail and broken tail (Figure 3).

Abnormality on spermatozoa tail is allegedly caused by lipid peroxidation. Silva (2006) said that mid-piece and posterior part of spermatozoa tail were very vulnerable to lipid peroxidation. Percentage of the lowest abnormality in this study was in the vitamin C administration. This showed that vitamin C played a role as a free-radical scavenger. Vitamin C protected lipid from oxidation reaction by extracellular (Fraga et al. 1991; Sitohang et al. 2015). It is possible due to the vitamin C administration may protect spermatozoa tail by extracellular that may prevent lipid peroxidation.

The integrity of plasma membrane (Table 7) of non-sexing spermatozoa without vitamin C (0%) was not

different with spermatozoa Y, but was significantly lower than spermatozoa X.

Administration of 0.25% or 0.5% vitamin C administration in non-sexing spermatozoa was not different with both spermatozoa X or Y, meanwhile, administration of 0.75% vitamin C in non-sexing spermatozoa was significantly lower than spermatozoa X and Y.

Non-sexed spermatozoa showed the same plasma membrane integrity in 0%, 0.25%, and 0.5% but significantly higher than 0.75% vitamin C administration.

Vitamin C administrations in spermatozoa X showed the same plasma membrane integrity in 0%, 0.25%, and 0.75%, but significantly lower than 0.5% vitamin C administration. Spermatozoa Y plasma membrane showed the same integrity in each vitamin C concentration.

Observation of plasma membrane integrity using hypoosmotic swelling (HOS) test marked by coiling tail for good plasma membrane and straight tail for the damage one (Figure 4). Plasma membrane served as physical protector of cell organelles and regulating exit and entry of nutrients and ions and maintaining electrolyte balance of extra and intra-cellulars (Sukmawati et al. 2014).

Percentage of plasma membrane integrity in this study (Table 7) increased by vitamin C administration. This proved that vitamin C was one of the vitamins serving as the soluble antioxidant which might prevent reactive oxygen activities with polyunsaturated fatty acids in the plasma membrane of spermatozoa (Wijaya 1995).

Research result in this study was in accordance with Sumargono (1998) who reported that administration of 1.5 mM vitamin C in tris-yolk might maintain integrity percentage of thawed spermatozoa plasma membrane of mud buffalo from damage due to lipid peroxidation. Besides, administration of 0.50 g/100 mL in Andromed diluent after thawing resulted percentage of plasma membrane integrity of Aceh cattle by 42.65±5.34% (Aslam et al. 2014). Difference of concentration of vitamin C used to integrity percentage of spermatozoa plasma membrane was allegedly due to difference in

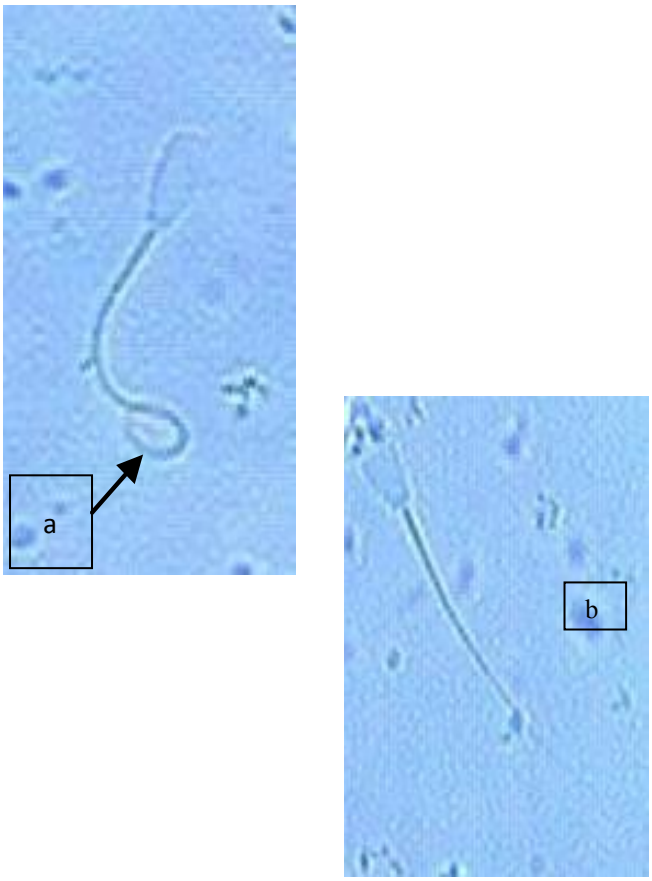


Figure 4. Integrity of plasma membrane of dairy cattle spermatozoa. (a) circle tail, good plasma membrane; (b) straight tail, damage plasma membrane.

used animal and diluent media. Toelihere (1993) explained that quality of spermatozoa was affected by diluent used.

Spermatozoa colored by acridine orange dye were used in observation of spermatozoa nucleus (Table 8). The acridine orange dye provided orange and yellow color in the dead or damage spermatozoa head and green color in alive or good spermatozoa head (Karezooni et al. 2009).

Integrity percentage of spermatozoa nucleus obtained in this study was in good condition (Figure 4). This proved that during sexing, freezing to thawing process did not cause damage in spermatozoa nucleus. Besides, high integrity percentage of spermatozoa nucleus in vitamin C treatment proved that vitamin C rolled as extracellular and intracellular free radicals scavenger (in the cytosol) (Padayatty 2003).

Observation in fresh semen and sexed spermatozoa is a basic to determine semen feasibility for further process. Komariah et al. (2013) reported quality of FH cattle spermatozoa before freezing collected from the bull in BIB Lembang (Table 9).

Table 9. Quality of FH cattle spermatozoa before freezing

Parameter	Criteria
Concentration	$1561x10^6$/ml
Mass Movement	Good
Motility	73.2%
pH	6.9

Source: Komariah et al. (2013)

Quality of thawed spermatozoa

Vitamin C was able to prevent damage to DNA caused by lipid peroxidation (Padayatty 2003). DNA damage caused by lipid peroxidation such as ring unveiling, fragmentation and DNA-protein cross-linking and DNA isolation would cause cells mutation or lethal (Awda et al. 2009). Besides, the result of lipid peroxidation such as malondialdehyde (MDA) and hydroxynonenol would trigger a modification of protein oxidation which was able to damage active sites enzyme, the conformation of protein structure and folding to form its original structure (Kumar et al. 2010; Susan & Rahayu 2013).

There was a quality decrease in non-sexing spermatozoa administered by 0.50% and 0.75% vitamin C and 0.75% vitamin C administration in spermatozoa X and Y. This is allegedly due to the decrease of pH. pH decrease in cell environment would disturb metabolic enzyme performance (Lehninger 1982). This is in accordance with the research results obtained by Sumargono (1998), Aslam et al. (2014) and Savitri et al. (2014). Sumargono (1998) said that motility of thawed mud buffalo spermatozoa decreased by administrating high vitamin C (3-5 mMolar diluent) due to the decrease of diluent pH. This is also in accordance with Aslam et al. (2014) who reported that administration of 2.00g/100ml vitamin C in Andromed diluent decreased the integrity of thawed spermatozoa plasma membrane of Aceh cattle. Administration of 4.50 mM vitamin C in diluent decreased the viability of thawed Bali cattle spermatozoa (Savitri et al. 2014).

The decrease of spermatozoa quality was also associated with the lactic acid increase in diluent media. Werdhany et al. (2000) said that longer thawed spermatozoa kept either at the time or before freezing (equilibration in 5°C) or after thawing would increase the number of dead spermatozoa due to the disability of media in maintaining pH which was more acid due to the accumulation of toxic lactic acid in spermatozoa.

CONCLUSIONS

Administration of vitamin C in diluent media improved the quality of non-sexing and X and Y spermatozoa of dairy cattle (*Bos taurus*) after thawing. The optimum concentration of vitamin C in diluent media to improve spermatozoa quality of sexed dairy cattle (*Bos taurus*) spermatozoa, both X and Y after thawing was 0.5%.

ACKNOWLEDGEMENTS

The authors wish to thank Meat and Milk Production project organized by Indonesian Research Institute for Biotechnology and Bioindustry for 2015 budget year and also to Tulus Maulana and Edy Sophian for helping in sample collection in this study.

REFERENCES

Afiati F. 2004. Proporsi dan karakteristik spermatozoa X dan Y hasil separasi kolom albumen. J Anim Sci Technol. 28:16-20.

Afiati A, Maulana T, Sophian E, Said S. 2014. Efektivitas penambahan vitamin C dalam medium pencucian terhadap kualitas sperma sapi. Pranoto Y, Udin LZ, Sardjono, Yusiati LM, Widyastuti Y, Aiman S, Hadiyanto, editors. Prosiding Seminar Nasional Sinergi Pangan, Pakan dan Energi Terbarukan. Yogyakarta (Indones): Indonesian Institute of Sciences. p. 299-303.

Agarwal A, Sekhon LH. 2010. The role of antioxidant therapy in the treatment of male infertility. Human Fertility. 13:217-225.

Anggraeny YN, Affandhy L, Rasyid A. 2004. Efektifitas substitusi pengencer Tris-Sitrat dan kolesterol menggunakan air kelapa dan kuning telur terhadap

kualitas semen beku sapi potong. Indones J Anim Vet Sci. 9:49-56.

Arifiantini I. 2012. Teknik koleksi dan evaluasi semen pada hewan. Bogor (Indones): IPB Press.

Aslam HA, Dasrul, Rosmaidar. 2014. Pengaruh penambahan vitamin C dalam pengencer andromed terhadap persentase motilitas dan membran plasmaa utuh spermatozoa sapi Aceh setelah pembekuan. J Med Vet. 8:20-26.

Awda JB, Mackenzie-Bell M, Buhr MM. 2009. Reactive oxygen species and Boar sperm function. J Bioreprod. 81:553-561.

[CSA] Central Statistic Agency. 2015. Statistik Indonesia 2015. Jakarta (Indones): Central Statistic Agency.

Franco JSV, Chaveiro A, Góis A, Da Silva FM. 2013. Effects of a-tocopherol and ascorbic acid on equine semen quality after cryopreservation. J Equine Vet Sci. 33:787-793.

Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacobs RA, Ames BN. 1991. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Medic Sci. 88:11003-11006.

Garner DL, Hafez ESE. 2000. Spermatozoa and seminal plasmaa. In: Hafez ESE, Hafez B, editors. Reproduction in Farm Animals. 7th ed. Philadelphia (USA): Lippincot Williams & Wilkins.

Hafez ESE, Hafez B. 2000. Reproduction in farm animal. 7th ed. South Carolina (USA): Lippincott Williams and Wilkins.

Hidayaturrahmah. 2007. Waktu motilitas dan viabilitas spermatozoa ikan Mas (*Cyprinus Carpio* L) pada beberapa konsentrasi larutan fruktosa. Bioscientiae. 4:9-18.

Hendri. 1992. Usaha megubah rasio sperma X dan Y dengan metode kolom menggunakan larutan Bovine Serum Albumin (BSA) dan penilaian angka kebuntingan serta perbandingan jenis kelamin anak pada kambing (Thesis). [Bogor (Indones)]: Institut Pertanian Bogor.

Kaiin EM, Afiati F, Gunawan M, Tappa B. 2003. Pengaruh penyimpanan pada temperatur 5°C terhadap viabilitas spermatozoa sapi hasil pemisahan. Mathius I-W, Setiadi B, Sinurat AP, Ashari, Darmono, Wiyono A, Purwadaria T, Murdiati TB, editors. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 73-76.

Karezooni T, Nasri A, Lella J, Marjaneh K, Alireza G, Fariborz G, Yasman K, Jaleh Z. 2009. Evaluation of sperm's chromatin quality with acridine orange test, chromomycin A3 and anline blue stainingincouples with unexpalined recurrent abortion. J Assist Reprod Genet. 26:591-596.

Komariah I, Arifiantini, Nugraha FW. 2013. Kaji banding kualitas spermatozoa sapi Simmental, Limousin dan Friesian Holstein terhadap proses pembekuan. Bul Peternak. 37:143-147.

Kumar V, Abbas AK, Fausto N, Aster JC, Perkins JA. 2010. Robbins and cotran pathology basic of disease, professional edition. 8th ed. Philadelphia (USA): Saunders Elsevier.

Lehninger AL. 1982. Dasar-dasar Biokimia. Jilid I. Thenawidjaja M, translater. Jakarta (Indones): PT Erlangga.

Lessard C, Parent S, Leclerc P, Bailey JL, Sullivan R. 2000. Cryopreservation alters the levels of the bull sperm surface protein P25b. Andrology. 21:700-707.

Ministry of Industry. 2013. Konsumsi susu masih 11,09 liter per kapita. [accessed April 22th 2012]. http://www.kemenperin.go.id/artikel/8890/Konsumsi-Susu-Masih-11,09-Liter-per-Kapita.

Moore AI, Squires EL, Graham JK. 2005. Adding cholesterol to the stallion sperm plasma membrane improves cryosurvival. Cryobiology. 51:241-249.

Nebel RL. 2007. Techniques for artificial insemination of cattle with frozen thawed semen. Current Therapy In Large Animal Theriogenol. 2nd ed. Missouri (USA): Saunders Elsevier.

Padayatty SJ. 2003. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. J Am Coll Nutr. 22:18-35.

Park JE, Graham JK. 1992. Effects of cryopreservation procedur on sperm membranes. Theriogenology. 38:209-222.

Pratiwi WC, Pamungkas D, Affandhy L, Hartati. 2006. Evaluasi kualitas spermatozoa hasil sexing pada kemasan straw dingin yang disimpan pada suhu 5°C selama 7 hari. Indones J Anim Vet Sci. 11:48-52.

Pratiwi WC, Affandhy L, Ratnawati D. 2011. Pengaruh lama *thawing* terhadap kualitas semen beku sapi Limousin dan Brahman. Anim Prod. 11:48-52.

Putra, Mahendra A, Susilawati T, Isnaini N. 2012. Kualitas dan proporsi spermatozoa X dan Y sapi Limousin setelah proses sexing menggunakan gradien densitas albumin putih telur. Malang (Indones): Universitas Brawijaya.

Salim MA, Susilawati T, Wahyuningsih S. 2012. Pengaruh metode *thawing* terhadap kualitas semen beku sapi Bali, sapi Madura dan sapi PO. Agripet. 12:14-19.

Savitri FK, Suharyati S, Siswanto. 2014. Kualitas semen beku sapi Bali dengan penambahan berbagai dosis vitamin C pada bahan pengencer skim kuning telur. J Ilm Peternak Terpadu. 2:30-36.

Silva PFN. 2006. Physiology of peroxidation processes in mammalian sperm (Thesis). [Utrecht (Netherland)]: Utrecht University.

Sitohang AG, Wantouw B, Queljoe E. 2015. Perbedaan antara efek pemberian vitamin C dan Vitamin E terhadap kualitas spermatozoa tikus wistar (Rattus norvegicus)

jantan setelah diberi paparan asap rokok. J E-Biomedik. 3:65-71.

Steel RGD, Torrie JH. 1995. Prinsip dan prosedur statiska. Sumantri B, translater. Jakarta (Indones): Gramedia Pustaka Utama

Subrata IM. 1999. Analisis sperma rutin. Denpasar (Indones): Upada Sastra.

Sugiarti T, Situmorang P, Triwulaningsih E, Kusumaningrum A, Lubis A. 2001. Pengaruh pemberian antioksidan dan prolin terhadap kualitas spermatozoa sapi setelah pembekuan. Haryanto B, Setiadi B, Sinurat AP, Mathius I-W, Situmorang P, Purwantari ND, Ashari, Abubakar, Murdiati TB, Hastiono S, Hardjoutomo S, Adjid RMA, Priadi A, editors. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 126-133.

Sukmawati E, Arifiantini RI, Purwantara B. 2014. Daya tahan spermatozoa terhadap proses pembekuan pada berbagai jenis sapi pejantan unggul. Indones J Anim Vet Sci. 19:168-175.

Sumargono T. 1998. Peningkatan spermatozoa kerbau lumpur dengan penambahan asam askorbat dalam pengencer semen beku (Thesis). [Bogor (Indones)]: Institut Pertanian Bogor.

Susan MD, Rahayu S. 2013. Profil protein spermatozoa setelah pembekuan. J Biotropika. 1:166-170.

Toelihere MR. 1993. Fisiologi reproduksi pada ternak. Bandung (Indones): Penerbit Angkasa.

Utomo B, Miranti DP. 2010. Tampilan produksi susu sapi perah yang mendapat perbaikan manajeman pemeliharaan. Caraka Tani. 25:21-25.

Werdhany IW, Toelihere MR, Supriatna I, Sutama I-K. 2000. Efek pemberian berbagai konsentrasi a-tokoferol sebagai antioksidan dalam pengencer Tris sitrat terhadap motilitas sperma kambing peranakan Etawah. Haryanto B, Murtiadi TB, Djajanegara A, Supar, Sutama I-K, Setiadi B, Darminto, Beriajaya, Abubakar, editors. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 244-252.

Wijaya A. 1995. Radikal bebas dan parameter status antioksidan. Jakarta (Indones): Prodia Diagnostics Education Services.

Winarto. 2010. Pemanfaatan vitamin E dan C sebagai antioksidan untuk memperbaiki kualitas dan kuantitas spermatozoa. [accessed March 5th 2015]. http://fapet-umpwr.blogspot.com/2010/03/.

Yuliani E, Lukman HY. 2013. Aplikasi sperma *sexing* berbasis antioksidan terhadap kual itas dan integritas membran serta daya fertilitas induk sapi Bali. Purwantari ND, Saepulloh M, Iskandar S, Anggraeni A, Ginting SP, Priyanti A, Wiedosari E, Yulistiani D, Inounu I, Bahri S, Puastuti W, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 23-30.

Productivity of *Indigofera zollingeriana* under Different Canopy and Soil Acidity Level in Oil Palm Estate

Herdiawan I

Indonesian Research Institue of Animal Production
Jl. Veteran III Banjarwaru Ciawi PO Box 221 Bogor Indonesia
E-mail: herdiawanmaliq@gmail.com

(received 11-02-2016; revised 27-05-2016; accepted 09-06-2016)

ABSTRAK

Herdiawan I. 2016. Produktivitas *Indigofera zollingeriana* pada berbagai taraf naungan dan kemasaman tanah di lahan perkebunan kelapa sawit. JITV 21(2): 135-143. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1361

Lahan perkebunan kelapa sawit di Indonesia sangat luas dan umumnya berada pada lahan sub-optimal yang berpeluang besar bagi pengembangan peternakan dalam penyediaan hijauan pakan. Penelitian bertujuan untuk mengetahui produktivitas *Indigofera zollingeriana* pada berbagai taraf naungan. Penelitian menggunakan rancangan RAK faktorial dengan 2 taraf perlakuan yaitu 3 taraf naungan umur kelapa sawit 2, 5 dan 7 tahun dan 2 taraf kemasaman tanah yaitu netral dan masam, masing-masing perlakuan diulang sebanyak 4 kali. Peubah yang diamati adalah produksi, dan kandungan nutrisi tanaman. Hasil penelitian menunjukkan tidak terdapat interaksi antara taraf naungan kelapa sawit dengan kemasaman tanah terhadap produksi segar daun, batang/ranting, biomasa, dan nisbah daun/ranting *I. zollingeriana*. Produksi segar daun, batang, biomasa, dan nisbah daun/ranting *I. zollingeriana* sangat nyata (P<0,01) menurun sejalan dengan taraf naungan. Kemasaman tanah nyata (P<0,05) menurunkan produksi segar daun, batang, biomasa, dan nisbah daun/ranting. Taraf perlakuan naungan nyata (P<0,05) meningkatkan kandungan protein kasar, serat kasar, dan energi, sebaliknya nilai kecernaan *in vitro* bahan kering dan bahan organik menurun. Kemasaman tanah nyata (P<0,05) menurunkan kandungan kasium, kecernaan *in vitro* bahan kering dan bahan organik *I. zollingeriana*.

Kata Kunci: *Indigofera zollingeriana*, Perkebunan Kelapa Sawit, Naungan, Tanah Masam

ABSTRACT

Herdiawan I. 2016. Productivity of *Indigofera zollingeriana* under different canopy and soil acidity level in oil palm estate. JITV 21(2): 135-143. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1361

Oil palm estate area in Indonesia is generally located in a sub-optimal land that has great opportunity for the development of forage supply. This study aims were to determine productivity of *Indigofera zollingeriana* under various canopy level. This research used factorial randomized block design with 3 canopy levels (under 2, 5, and 7 year oil palm canopy) and 2 levels of soil acidity (neutral and acid soil) treatments, where each treatment was repeated 4 times. Parameters observed were production and nutrient content of *Indigofera zollingeriana*. Research results showed that there was no interaction between the canopy levels and soil acidity on the production of fresh leaves, stems/branches, biomass, and leaves/stem ratio of *I. zollingeriana*. Production of fresh leaves, stems, biomass, and leaves/branches ratio of *I. zollingeriana* significantly (P<0.01) decreased along with increase of canopy level. Soil acidity significantly (P<0.05) decreased production of fresh leaves, stems, biomass, and leaves/branches ratio. Level of canopy treatment significantly (P<0.05) increased content of crude protein, crude fiber and energy, otherwise value of *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) were decrease. Soil acidity significantly (P<0.05) decreased calcium content, *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) of *I. zollingeriana*.

Kata Kunci: *Indigofera zollingeriana*, Oil Palm Estate, Canopy, Acid Soil

INTRODUCTION

Limited forage land in Indonesia is caused by several factors such as limited land availability, competition with other use, and high land convertion number. Mulyani et al. (2011) said that most of the remaining land for forthcoming agricultural development was sub-optimal or marjinal land, such as rainfed land; acidic dryland and wetland with various biosfic issues. Atman (2006) reported that most of total area available in Indonesia (190,946,500 ha) for agriculture area were clasified as Ultisol or acidic dryland. Ultisol soil was drysoil with high abiotic stresses, such as soil pH <4, organic content, low cation exchange capacity, and high Mn^{2+} and reactive aluminium (Al^{3+}) element which was able to poison plant root and preventing root nodule formation in legume (Hairiah et al. 2006). Then Subagyo et al.

(2004) said that Ultisol land was more widely used for estate and protected forest area due to its high abiotic stresses. Nowadays, those lands were mostly used for oil palm estate around 5.3 million ha (CSA 2012). Therefore, oil palm estate is one of forage land resources.

Cultivation of forage in oil palm estate areal faces 2 main problems, that are low sunlight intensity and hight soil acidity stresses. Light intensity under forest vegetation largerly determines process of photosystesis, botanical composition, growth, and quality of forage nutrition available for ruminant (Blair et al. 1983). As Das et al. (2008) said that forage cultivation under oil palm estate areal was restricted by low soil pH and sunlight intensity along with oil palm growth decreasing forage production. Physiologically, canopy will decrease sunlight intensity required for assimilation process of plants below. Crowder & Chheda (1982)said that decrease of incoming sunlight intensity significantly increased assimilation rate and CO_2 income decreasing quality and quantity of canopy-underneath plants. Wilson & Ludlow (1991) described that shading rate of estate plants canopy might reach 80% depending on variety of plant, plant spacing and age.

Wong & Chin (1998) said that underneath-forage production decreased along with oil palm aged. Along with oil palm aged, sunlight penetrating oil palm leaves was getting low affecting production of dry material production of the underneath plants. Chin (1998) said that dry material production of forage under nursling oil palm plant might reach 1600-2600 kg/ha and decreased to 600 kg/ha along with oil palm aged. Low transmission affected microclimate under canopy and then decreasing soil temperature. This condition might prevent growth and dry material accumulation of plants growing under oil palm trees (Abdullah 2011). Horne (1994) described that there were 2 ways to improve and increase quality and production of forage under oil palm and rubber estates. One of them was ntroduction of shade-tolerant forage to support its sustainable production. Therefore, technologies of cultivation in certain oil palm age and shade-tolerant forage in such specific condition were required, so that productivity of forage positively contributed to the both sides.

Based on research results conducted in a greenhouse, *I. zollingeriana* had high tolerant against acidic soil stress than *C.calothyrsus* and *G. sepium* (Herdiawan & Sutedi 2013). Subject of this study was to determine productivity of *I. zollingeriana* in acid soil condition and under oil palm canopy level to support oil palm-cattle integration.

MATERIALS AND METHODS

This study was conducted in oil palm estate in Deli Serdang district, North Sumatra by planting *I. zollingeriana* as an intercropping plant. Preparation step was conducted by sowing seed in seeding tray containing of 1 : 1 soil and compose until 4 weeks, and than moved to small polybag until 8 weeks old. Eight weeks old plants were moved to field by 2x2 m row spacing and each plot size was 8x30 m. Planting was conducted between 2, 5, and 7 years old trees in acid and neutral soil condition by administratin super dolomite (5 ton/ha). This study used factorial Randomized Block Design (Gomez and Gomes, 1984) with 3 canopy levels and 2 soil acidity levels with 4 repetitions. Based on Solarimeter, average light intencities in 2 (control), 5, and 7 years oil palm estate were 2632.90 cal/m^2, 1751.30 cal/m^2 and 698.70 cal/m^2, respectively. Soil acidity was assessed using pH tester and lacmus paper to soil administered by super dolomite (pH 4.72). First pruning was conducted in 60 DAP (days after planting) and then harvested 1 meter above the ground in every 90 days. Parameters observed were production of biomass, leaves, brances, ration leaves/brances and nutrients content (CP, CF, Energy, Ca, P, *in vitro* digestibility of dry and organic materials)

RESULTS AND DISCUSSION

Production of *I. zollingeriana*

Based on analysis of variance, there was no interaction between canopy level and soil acidity to fresh biomass production of *I. zollingeriana* (Table 1).

Table 1. Fresh biomass production of *I. zollingeriana* in various canopy levels and soil acidity (g/plant) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	6701.59	1020.00	355.11	2692.23[a]
Acid	6645.24	92318	353.13	2640.52[a]
Average	6673.42[a]	971.59[b]	354.12[c]	

The different letters in column and row shows significant difference (P<0.01)

Table 2. Average production of fresh leaves of I. zollingeriana in various canopy levels and soil acidity (g/plant) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	2800.30	301.17	97.08	1066.18[a]
Acid	2772.16	271.06	69.46	1037.56[b]
Average	2785.23[a]	286.12[b]	83.27[c]	

The different letters in column and row shows significant difference (P<0.01)

Production of fresh biomass under 2 year oil palm canopy was significantly higher by 6701.59 g/plant. The lowest production of fresh biomass was under 7 years oil palm canopy by 353.13 g/plant. Meanwhile, administration of super dolomite was not significantly different. Total production of plants and roots of all plants was influenced by canopy, where production of plants under canopy was very low followed by production of uper-part biomass (Congdon & Addison 2003). It was reported that average production of tropical forage biomass without canopy (control) was 40.11 kg/pot and decreased in 63% canopy level into 18.99 kg/pot. It sharply decreased from 76% and 84% into 7.08 and 6.27 kg/pot, respectively.

Farizaldi (2011) reported that production of forage dry material either grass and legume under 8 years oil palm trees was lower than in 5 and 3 years. This low production was caused by low light intencity due to bigger canopy shape along with oil palm aged. Batubara et al. (1999) said that older oil palm tree required more light, water and nutrient, so that its availability for underneath-plants was decrease. Average production of grass under 5-10 years oil palm trees was 10.479 ton/ha/year and increased into 14.827 ton/ha/year in 10-20 years oil palm trees. Older oil palm trees had less canopy level, so that it received more light than the 5-10 years oil palm trees. Hanafi et al. (2005) reported his research results showing that production of fresh forage planting by monoculture under 55% canopy level of oil palm was (5890.73 kg/ha) better than under 75% canopy level (5347.26 ton/ha). Production of fresh forage per m^2 of vegetation growing under 3 and 6 years oil palm trees was 386.54 g/m^2 and 189.29 g/m^2, respectively (Daru et al. 2014). Production of *Indigofera zollingeriana* biomass under 5 years oil palm trees was higher than native grasses under the same age of oil palm trees. Then there was a significant decrease of *Indigofera zollingeriana* production under 7 years oil palm trees.

Based on analysis of variance, there was no interaction between canopy level of oil palm and super dolomite adiminstration to production of fresh leaves of *I. zollingeriana* (Table 2). This was inaccordance with Jaramillo et al. (2010) who said that there was no significant interaction between canopy level and limestone application to production and leaves surface area per brance.

Production of fresh leaves under 2 years oil palm trees was the highest (P<0.01) by 2800.30 g/crop. The lowest production of fresh leaves was under 7 years oil palm trees by 69.46 g/crop. Canopy level and soil acidity significantly affected production of *I. zollingeriana* fresh leaves.

Dı´az-Pe´rez (2013) reported that weight of leaves, stems and upper biomass were significantly different among the canopy level treatment. It was also reported that canopy changed plant morphologically with thiner and wider leaves and lighter weight. Atwell et al. (1999) also reported that plants growing under canopy would show horizontal adaptation response and smaller chloroplast. Canopy level by 40% decreased tomato leaves weight by 24% than the plants without canopy (Bertin & Gary 1998). Then, Qifu et al. (2002) said that A1 content in high acid soil might disturb soy growth and ruined plant roots leading to low production of plant due to inefficiency of nutrient and water absorption by roots. Chen et al. (2005) said that A1 decreased CO_2 intake useful in assimilation of tangerine (*Citrus rehhni*) affecting enzyme activities involved in Calvin cycle. Nutrients supply decreased by the assimilation process disruption decreasing production and quality of plants, especially in plants sensitive to A1 stress. Hilman et al. (2004) said that in acid dry land, phosphate (P) availability was the main issue in increasing legume production.

Analysis of variance showed no interaction between canopy level of oil palm and soil acidity to production of fresh stems/branches of *I. zollingeriana* (Table 3).

The highest (3887.19 g/crop) production of fresh stems/braches was under 2 years oil palm trees and the lowest production by 270.85 g/plant was under 7 years oil palm trees. Meanwhile, administration of super dolomite was not significantly affected production of stems/branches of *I. zollingeriana*. Stems diameter shaded was thiner due to elongated growth than unshaded plants which affecting stems biomass. Larcher (1995) also said that stems diameter related to dry weight of upper plant, leaves area, and plant

Table 3. Production of fresh stems/branches of *I. zollingeriana* in various canopy levels and soil acidity (g/plant) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	3901.29	718.83	258.03	1626.05[a]
Acid	3873.08	652.12	283.67	1602.96[a]
Average	3887.19[a]	685.48[b]	270.85[c]	

The different letters in column and row shows significant difference (P<0.01)

Table 4. Ratio of leaves/branches of *I. zollingeriana* in various canopy levels and soil acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	0.72	0.42	0.38	0.51[a]
Acid	0.72	0.42	0.46	0.53[a]
Average	0.72[a]	0.42[b]	0.31[c]	

The different letters in column and row shows significant difference (P<0.01)

ability to carry water from soil to leaves. Wilson & Ludlow (1991) said that morphological responses of plants under canopy such as stems extention and branching reduce might decrease dry material production due to fewer axillary buds and same leaves area. Research resulted by Kittas et al. (2012) showed that chili plant under canopy had long stems, wider and thiner leaves and low leaves weight.

Research results showed no interaction between canopy level of oil palm and soil acidity to ratio of leaves/branches of *I. zollingeriana*. Ratio of leaves/branches under 2 years was significantly (P<0.01) higher by 0.72 than under 5 and 7 years by 0.42 and 0.31, respectively. Ratio of leaves/branches in neutral and acid soil did not different (Table 4).

Shehu et al. (2001) said that ratio of leaves/branches was highly crucial since it was a metabolic organ and affected quality of legume. More leaves number showed better quality of legume. Leaves consisted highest nutrients than stems/branches. Ratio of crown/roots increased in plants under canopy due to increase of proportion of crown by sacrificing rooting system to obtain sufficient sunlight for assimilation process (Atwell et al. 1999). Alocation of resources excessively from roots to bud might lead susceptibility of plants to water stress periodically and intensive pruning. Too high root system decrease led decrase of DM production and longer recovery periode, where regrowth after defoliation related to carbohydrate and mineral reserves in root (Wilson & Ludlow 1991). Karim et al. (1991) said that increasing plant age resulted lower ratio of leaves and branches. This low ratio affected crude protein and energy content. The most protein and energy was in branches, higher leaves ratio than branches produced higher protein and energy content which was crucial in animal productivity.

Nutrient content of *I. zollingeriana*

Based on analysis of variance, there was no interaction between oil palm canopy and soil acidity to crude protein content of *I. zollingeriana* (Table 5). Crude protein content of plnats under 2 years oil palm canopy was significantly higher (P<0.05) by 26.99% than that under 5 and 7 years oil palm by 23.15% and 25.61%, respectively. Crude protein content in neutral and acid soil was not different. Daru et al. (2014) reported that crude protein content of plant under 6 years oil palm canopy was higher than that wthout canopy. Canopy influenced forage quality either directly or indirectly changing chemical composition. Then, Wilson & Wild (1995) said that N concentration in leaves consistently was higher under canopy than the one without canopy. Generally, there was high increase of N concentration in leaves in canopy treatment by 63% compared to the one without canopy. After all, canopy increase up to 76 and 84% was slighty increased N in leaves, but not in previous level.

N concentration of plant materials under canopy generally increased (Humphreys 2005). Congdon & Addison (2003) said that N concentration in leaves was greatly influenced by canopy, where its concentration increased in under canopy than control, but there was no significantly change in P concentration in leaves. Kephart & Buxton (1993) said that concentration of

Table 5. Crude protein content of *I. zollingeriana* in various canopy levels and soil Acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	23.94	25.20	26.83	24.99[a]
Acid	22.35	26.01	27.14	24.48[a]
Average	23.15[c]	25.61[b]	26.99[a]	

The different letters in column and row shows significant difference (P<0.01)

Table 6. Crude fiber content of *I. zollingeriana* in various canopy levels and soil acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	14.76	17.10	17.68	16.51[a]
Acid	12.37	18.22	18.12	16.24[a]
Average	13.57[c]	17.66[b]	17.90[b]	

The different letters in column and row shows significant difference (P<0.05)

crude protein was much more responsive to canopy compared to other quality components. It was also said that 63% canopy might increase crude protein concentration by 26% in grass. Norton et al. (1990) said that forage grown under canopy had higher nitrogent content than forage grown on the open field. High nitrogent content was caused by canopy ease nitrogent availability in soil to be absorbed by plant and then increasing nitrogent content in plant tissue (Wilson & Ludlow 1991; Wong & Wilson 1980). Yayneshet et al. (2009) reported that crudeprotein content from forage in semi-arid area in Ethiopia drastically decreased which was caused by dry and soil acidity stresses. Higher structural component (NDF, ADF and ADL) content found during dry season especially in acid soil was alegedly due to high lignification and maturiry stadium of plant (Hussain & Durrani 2009). Khan et al. (2008) said that overuse of organic fertilizer would damage soil structure, increase soil acidity, cause nutritional imbalance, and decrase production and quality of plant.

Based on analysis of variance, there was no interaction between oil palm canopy and soil acidity to crude fiber content of *I. zollingeriana* (Table 6). Crude fiber content under 2 years oil palm trees was significantly (P<0.05) lower by 13.57% than under 5 and 7 years oil palm treatment by 17.66 and 17.90%, respectively. Crude fiber under 5 and 7 years oil palm trees did not different significantly. Then crude fiber content in neutral and acid soil did not show a difference. Blair et al. (1983) reported than crude protein content and cell wall consistency such as ADF and cellulose increased along with canopy density increase. Humphreys (2005) also said that canopy would change quality of light spectrume which would be up on the leaf surface affecting in tiller and germanisation. Light, one of components of photosynthesis process conversed carbone monoxide and water into glucose and structure carbone forming cell wall, cellulose and hemicellulose. Decrease of light intencity did not affect lygnin level, however the highest lygnin content was schieved in dense canopy shade (Blair et al. 1983).

There was no interaction between oil palm canopy and soil acidity to energy content of *I. zollingeriana* (Table 7). Energy content under 7 years oil palm treatment was significantly (P<0.05) higher by 4015.0 Kcal/kg than under the 2 and 5 years oil palm treatment by 3749.0 and 3895.3 Kcal/kg, respectively. Energy content in neutral was significantly lower by 3790.3 Kcal/kg than in acid soil by 3982.5 Kcal/kg. Increase of canopy level increases forming of structure carbone in plant cell wall increasing crude fiber content. Energy was a metabolism product of energy resource foods such as carbohydrate including crude fiber, cellulose, hemicellulose and lygnin digested by ruminal microbes in the digestive tract (Dewhurst et al. 2009). Energy producted from that metabolic process was used for maintenance; growth and production of milk, meat, egg, and wool (William 2010). Gross energy was one of crude fiber methabolic products in ruminant digestive tract with ruminal enzyme and microbes help. As reported by Dewhurst et al. (2009) that increase of gross energy of forage was alwasy in line with increase of crude fiber of dry material of forage especially cellulose component which ease to be hydrolized by acid or cellulose enzyme resulted by ruminal microorganism into monomer glucose.

Table 7. Energy content of *I. zollingeriana* in various canopy levels and soil acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	3406.0	3875.0	4090.0	3790.3[b]
Acid	4384.5	3623.0	3940.0	3982.5[a]
Average	3895.3[b]	3749.0[c]	4015.0[a]	

The different letters in column and row shows significant difference (P<0.05)

Table 8. Calcium (Ca) content of *I. zollingeriana* in various canopy levels and soil acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	0.92	0.94	1.71	1.19[a]
Acid	0.78	0.81	0.90	0.83[b]
Average	0.85[b]	0.88[b]	1.31[a]	

The different letters in column and row shows significant difference (P<0.05)

Karim et al. (1991) reported that increase of plant age and dry stress decreased ratio of leaves/stems, meanwhile increased ratio of stems/leaves. This decreased crude protein content, but instead increased gross energy. The most gross energy in plant is in stem due to carbohydrate content in the form of crude fiber (cellulose, hemicellulose and lignin).

There was no interaction between oil palm canopy and soil acidity to calcium (Ca) content of *I. zollingeriana* (Table 8). Calcium (Ca) content under 7 years oil palm trees was significantly (P<0.05) higher by 1.31% than under the 2 and 5 years oil palm trees by 0.85 and 0.88%, respectively, however there was no significant difference between 2 and 5 years oil palm canopy treatment. Then, Ca content in neutral soil was significantly higher (P<0.05) by 1.19% than in acid soil by 0.83%. Blair et al. (1983) said that concentration of phosphor and calcium was significantly higher under dense canopy than under medium canopy and without canopy.

There was no interaction between oil palm canopy and super dolomit administration to phosphor of *I. zollingeriana* (Table 9). There was no significant difference between canopy level under 2, 5, and 7 years oil palm to phosphor content, as well as in soil acidity level. Blair et al. (1983) reported that phosphor (P) and calcium (Ca) concentration was significantly higher under dense canopy than under medium canopy and without canopy. Congdon & Addison (2003) said that N concentration in leaves was greatly influenced by canopy, where its concentration increased under canopy than control, whereas it did not change significantly to concentration of phosphor in leaves.

Based on analysis of variance, there was no interaction between oil palm canopy and soil acidity to dry material digestibility of *I. zollingeriana* (Table 10). Digestibility of dry material under 2 years oil palm trees was significantly (P<0.05) higher by 71.53% than under 5 and 7 years oil palm trees by 65.45 and 62.78 %, respectively. It was significantly higher by 67.20% in neutral soil than in acid soil by 65.98%.

Digestibility of dry material decreased, because closer canopy would increase crude fiber in plant. Blair et al. (1983) reported that dry material digestibility was very good under full sunlight or medium canopy. Dry material digestibility by *in vitro* was the number of digestable and not excreted dry material in the form of faecess and was assumed as a part absorbed by animal (Chuzaemi & Bruchem 1990). One of the reasons of low dry material digestibility was high lygnin content in skin cell wall of plant which might prevent enzyme to normaly digest fiber. Sleugh et al. (2001) reported that decrease of dry material digestibility was in line with frequency of prunning due to accumulation of indigestible fiber, lignification increase and decrease of leaves/branches ratio, would form cell wall structure making it difficult to be digested by ruminal microbes. Digestibility value of grass and legume, generally decreased along with plants aged and decrease of soil water content due to increase of crude fiber concentration in plant tissue, increase of lignification and decrease of ratio leaves/stems (Nisa et al. 2004).

Table 9. Phospor content *I. zollingeriana* in various canopy levels and soil acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	0.28	0.27	0.27	0.27[a]
Acid	0.26	0.26	0.26	0.26[a]
Average	0.27[a]	0.27[a]	0.27[a]	

The different letters in column and row shows significant difference (P<0.05)

Table 10. Digestibility of dry material of *I. zollingeriana* in various canopy level and soil acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	70.65	67.65	63.29	67.20[a]
Acid	72.41	63.25	62.27	65.98[b]
Average	71.53[a]	65.45[a]	62.78[c]	

The different letters in column and row shows significant difference (P<0.05)

Table 11. Digestibility of organic material of *I. zollingeriana* in various canopy levels and soil acidity (%) of oil palm estate

Soil acidity	Canopy level (age of oil palm)			Average
	2 years	5 years	7 years	
Neutral	70.16	63.65	60.32	64.71[a]
Acid	68.62	61.25	60.86	63.58[b]
Average	69.39[a]	62.45[b]	60.59[c]	

The different letters in column and row shows significant difference (P<0.05)

Based on analysis of variance, there was no interaction between oil palm canopy and soil acidity to organic material digestibility of *I. zollingeriana* (Table 11). Organic material digestibility under 2 years oil palm trees was significantly higher by 69.39% than those under 5 and 7 years oil palm trees by 62.45 and 60.59%, respectively. Then organic material digestibility in super dolomit administration treatment was significantly (P<0.05) higher by 64.71% than in soil without super dolomit by 63.58%.

Digestibility of organic material of forage was organic material value including crude protein, carbohydrate, fiber to digest and not excreted through faecess and might be used as indicator of overall forage quality. Low digestibility of organic material as well as dry material digestibility was caused by high crude fiber, expecially lygnin in its basic material of forage. The highest and lowest digestibility of organic material of *I. zollingeriana* by 76.02% and 63.86%, respectively was still higher than digestibility of *Gliricidia sepium* by 60.82% (Sánchez et al. 2005). González & Hanselka (2002) said that digestibility of organic material of forage decreased significantly from rainy to dry season in line with increase of some crude fiber-forming components. (Hassen et al. 2007) stated that all of *Indigofera* species had higher ash, crude protein, and organic material digestibility with lower NDF concentration in spring.

CONCLUSION

Denser canopy level of oil palm (5 and 7 years old) significantly decreased fresh production of *I. zollingeriana* either in netral and acid soil. Nutrient content of CP, CS, energy, Ca and P of *I. zollingeriana* increased along with level increase of oil palm canopy shade (5 and 7 years old), otherwise digestibily of dry and organic material were decrease. *I. zollingeriana* was not tolerant to dense oil palm canopy (5 and 7 years old), but had better quality and quantity in lesser canopy dense (2 years old).

REFERENCES

Abdullah L. 2011. Prospek integrasi perkebunan kelapa sawit-sapi potong dalam upaya percepatan pencapaian swasembada daging nasional 2014: Sebuah tinjauan perspektif penyediaan pakan. Sangatta (Indones): Sekolah Tinggi Ilmu Pertanian Kutai Timur.

Atman. 2006. Pengelolaan tanaman kedelai di lahan kering masam. J Ilm Tambua. 5:281-287. doi: 10.1080/14620316.2012.11512822.

Atwell B, Kriedemann P, Turnbull C. 1999. Plants in action - adaptation in nature, performance in cultivation. South Yarra (Aust): McMillan Education Australia Pty., Ltd.

Batubara A, Kasup I, Kesma AA, Irfan A, Simanjuntak H, Harahap P. 1999. Kajian integrasi penggemukkan ternak sapi potong di lahan perkebunan kelapa sawit. Bogor (Indonesia): Balai Penelitian Ternak.

Bertin N, Gary C. 1998. Short and long term fluctuations of the leaf mass per area of tomato plants - Implications for growth models. Ann Bot. 82:71-81. doi: 10.1006/anbo.1998.0647.

Blair RM, Rene A, Austin H. 1983. Shade intensity influences the nutrient quality and digestibility of southern deer browse leaves. J Range Manag. 36:257-263. doi: 10.2307/3898177.

[CSA] Central Statistic of Agency. 2012. Statistik Indonesia 2012. Jakarta (Indones): Central Statistic of Agency.

Chen LS, Qi YP, Smith BR, Liu XH. 2005. Aluminum-induced decrease in CO_2 assimilation in citrus seedlings is unaccompanied by decreased activities of key enzymes involved in CO_2 assimilation. Tree Physiol. 25:317-324.

Chin FY. 1998. Sustainable use of ground vegetation under mature oil palm and rubber trees for commercial beef production. de la Vina AC, Moog FA, editors. Proceeding of 6th Meeting Region Work Grazing Feed Resources Southeast Asia. Rome (Italy): Food Agriculture of Organization. p. 35-44.

Chuzaemi S, Bruchem JV. 1990. Fisiologi nutrisi ruminansia. KPK UGM-Brawijaya. Program Pascasarjana. Spesialisasi pakan ternak. Malang (Indones): Universitas Brawijaya.

Congdon B, Addison H. 2003. Optimising nutrition for productive and sustainable farm forestry systems pasture legumes under shade. A report for the RIRDC/Land & Water Australia/FWPRDCMDBC. Joint Venture Agroforestry Program. Townsville (Aust): James Cook University.

Crowder LV, Chheda HR. 1982. Tropical grassland husbandry. New York (USA): Longman Group.

Daru TP, Yulianti A, Widodo E. 2014. Potensi hijauan di perkebunan kelapa sawit sebagai pakan sapi potong di kabupaten Kutai Kartanegara. Media Sains. 7:79-86.

Das DK, Chaturvedi OP, Mandal MP, Kumar R. 2008. Effect of tree plantations on biomass and primary productivity of herbaceous vegetation in eastern India. Trop Ecol. 49:95-101.

Dewhurst RJ, Delaby L, Moloney A, Boland T, Lewis E. 2009. Nutritive value of forage legumes used for grazing and silage. Ir J Agric Food Res. 48:167-187.

Dı´az-Pe´rez JC. 2013. Bell pepper (*Capsicum annum* L.) Crop as affected by shade level: microenvironment, plant growth, leaf gas exchange, and leaf mineral nutrient concentration. HortScience. 48:175-182.

Farizaldi. 2011. Produktivitas hijauan makanan ternak pada lahan perkebunan kelapa sawit berbagai kelompok umur di PTPN 6 Kabupaten Batanghari Propinsi Jambi. J Ilmu Peternak. 11:75-78.

González VEA, Hanselka CW. 2002. Ecología y manejo de matorrales. Caso provincia biótica tamaulipeca. Mexico (USA): INIFAP-Texas A&M University, Social and Comercial Press.

Hairiah K, Rahayu S, Berlian. 2006. Layanan lingkungan agroforestri berbasis kopi: Cadangan karbon dalam biomasa pohon dan bahan organik tanah (studi kasus dari Sumberjaya, Lampung Barat). Agrivita. 28:298-309.

Hanafi ND, Umar S, Bachari I. 2005. Pengaruh tingkat naungan pada berbagai pastura campuran terhadap produksi hijauan. J Agribisnis Peternak. 1:100-105.

Hassen A, Rethman NFG, van Niekerk WA, Tjelele TJ. 2007. Influence of season/year and species on chemical composition and *in vitro* digestibility of five *Indigofera* accessions. J Anim Feed Sci Technol. 136:312-322. doi: 10.1016/j.anifeedsci.2006.09.010.

Herdiawan I, Sutedi E. 2013. Productivity of Calliandra calothyrsus, Indigofera zollingeriana and Gliricidia sepium on acid soil in the greenhouse. Indones J Anim Vet Sci. 20:105-114. doi: 10.14334/jitv.v20i2.1165.

Hilman Y, Kasno A, Saleh N. 2004. Kacang-kacangan dan umbi-umbian: Kontribusi terhadap ketahanan pangan dan perkembangan teknologinya. In: Makarim, editor. Inovasi pertanian tanaman pangan. Bogor (Indones): Indonesian Center for Food Crops Research and Development. p. 95-132.

Horne PM. 1994. Agroforestry plantation system□: Sustainable forage and animal in rubberand oil palm plantation. In: Copland JW, Djajanegara A, Sabrani M, editors Agroforestry and Animal Production for Human Welfare at 7th Animal Science Congress of Asia-Australasian Animal Production. Bali (Indonesia): ACIAR.

Humphreys LR. 2005. Tropical pasture utilitisation. Cambridge (UK): Cambridge University Press.

Hussain F, Durrani MJ. 2009. Nutritional evaluation of some forage plants from Harboi rangeland, Kalat, Pakistan. Pak J Bot. 41:1137-1154.

Jaramillo BC, Santos RHS, Martinez HEP, Fardin MP. 2010. Production and vegetative growth of coffee trees under

fertilization and shade levels. Sci Agric. 67:639-645. doi: 10.1590/S0103-90162010000600004.

Karim AB, Rhodes ER, Savill PS. 1991. Effect of cutting interval on dry matter yield of *Leucaena leucocephala* (Lam) De Wit. Agrofor Syst. 16:129-137. doi: 10.1007/BF00129744.

Kephart KD, Buxton DR. 1993. Forage quality responses of C_3 and C_4 perennial grasses to shade. Crop Sci. 33:831-837.

Khan HZ, Malik MA, Saleem MF. 2008. Effect of rate and source of organic material on the production potential of spring maize (*Zea mays* L.). Pak J Agric Sci. 45:40-43.

Kittas C, Katsoulas N, Rigakis N, Bartzanas T, Kitta E. 2012. Effects on microclimate, crop production and quality of a tomato crop grown under shade nets. J Hortic Sci Biotechnol. 87:7-12.

Larcher W. 1995. Physiological plant ecology. Ecophysiological and stress physiology of functional groups. In: Demming-Adams B, Adams III WW, Borchert R, van Bel AJE, Ernst WHO, Kappen L, Korner C, Kuppers M, Ma JF, Richter H, et al., editors. 4th ed. Berlin (Germany): Springer.

Mulyani A, Ritung S, Las I. 2011. Potensi dan ketersediaan sumber daya lahan untuk mendukung ketahanan pangan. J Litbang Pertan. 30:73-80.

Nisa M, Sarwar M, Khan MA. 2004. Nutritive value of urea treated wheat straw ensiled with or without coern steep liquor for lactating Nili-ravi buffaloes. Asia-Austral J Anim Sci. 17:825-829. doi: 10.5713/ajas.2004.825.

Norton BW, JR Wilson JR, Shelton HM, Hill KD. 1990. Urea treated wheat straw with or without corn steep liquor on feed consumption, digestibility and milk yield and its composition in lactating Nili-ravi buffaloes. Forages for plantation crops. In: Proceeding Work. Bali (Indones); p. 83-88.

Qifu MA, Rengel Z, Kuo J. 2002. Aluminium toxicity in rye (*Secale cereale*): root growth and dynamics of cytoplasmic Ca^{2+} in intact root tips. Ann Bot. 89:241-244.

Sánchez MI, Green AJ, Castellanos EM. 2005. Seasonal variation in the diet of the redshank Tringa totanus in the Odiel Marshes, south-west Spain: A comparison of faecal and pellet analysis. Bird Study. 52:210-216. doi: 10.1080/00063650509461393.

Shehu Y, Al-Hassan WS, Pal UR, Philips CSJ. 2001. Yield and chemical composition response of *Lablab purpureus* to nitrogen, phosphorus and potassium fertilizers. Trop Grassl. 35:180-185.

Sleugh BB, Moore KJ, Brummer EC, Knapp AD, Russell J, Gibson L. 2001. Forage nutritive value of various Amaranth species at different harvest dates. Crop Sci. 41:466-472.

Subagyo H, Suhartab N, Siswanto AB. 2004. Tanah-tanah pertanian di Indonesia. Dalam: Sumberdaya lahan Indonesia dan pengelolaannya. Adimihardja A, Amien LI, Agus F, Djaenudin D, editors. Bogor (Indones): Indonesian Center for Agricultural Land Resources Research and Development.

William PW. 2010. Refining the net energy system. WCDS Adv Dairy Technol. 22:191-202.

Wilson JR, Ludlow MM. 1991. The environment and potential growth of herbage under plantations. Stur WW, editor. ACIAR Proceeding of Forages Plant Crop. Canberra (Aust): Australian Centre for International Agricultural Research. p. 10-24.

Wilson JR, Wild DWM. 1995. Nitrogen availability and grass yield under shade environments. In: Mullen BF, Shelton HM, editors. ACIAR 64 - Integration Ruminants into Plant System in Southeast Asia. Canberra (Aust): Australian Centre for International Agricultural Research.

Wong CC, Chin FY. 1998. Meeting nutritional and requirement of cattle from natural forages in oil plantation. In: Proceeding of National Seminar Livestock Crop Integration Oil Palm-Toward Sustainable. Johor (Malays): Palm Oil Research Institute of Malaysia. p. 10-19.

Wong CC, Wilson JR. 1980. Effect of shading on growth and nitrogen content of green panic and siratro in pure and mixed swards defoliated at two frequencies. Aust J Agric Res. 31:269-185.

Yayneshet T, Eik LO, Moe SR. 2009. Seasonal variations in the chemical composition and dry matter degradability of enclosure forages in the semi-arid region of Northern Ethiopia. J Anim Feed Sci Technol. 148:12-33.

Determination of Fibernolytic Enzyme Activities of White Rot Fungi Isolated from Oil Palm Fronds

Azmi MAB[1], Alias S[1], Azmi AFM[1], Ghani AAA[1], Shahudin MS[1], Goh YM[1], Noordin MM[1], Yusof MT[2], Zunita Z[1], Hassim HA[1]

[1]*Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia*
[2]*Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia*
E-mail: haslizaabu@upm.edu.my

(received 10-05-2016; revised 26-06-2016; accepted 16-05-2016)

ABSTRAK

Azmi MAB, Alias S, Azmi AFM, Ghani AAA, Shahudin MS, Goh YM, Noordin MM, Yusof MT, Zunita Z, Hassim HA. 2016. Penentuan aktivitas enzim pengurai serat kapang lapuk putih yang diisolasi dari pelepah sawit. JITV 21(2): 144-150. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1362

Pelepah sawit (OPF) banyak digunakan sebagai sumber serat untuk hewan ternak. Namun, potensi keseluruhan OPF sebagai pakan ternak dibatasi oleh kadar lignin yang tinggi yang menghambat aktivitas rumen mikroba untuk menguraikan selulosa dan hemiselulosa. Fungi lapuk putih (WRF) adalah kelompok fungi filum basidiomisetes, umumnya ditemukan di kayu busuk, dan mampu untuk mendegradasi lignin. Percobaan ini bertujuan untuk mengidentifikasi filum fungi yang terbaik berdasarkan aktivitas rasio enzim pengurai lignin terhadap selulase dan hemiselulase. Dalam percobaan ini, sebelas isolat fungi didapatkan dari pelepah sawit busuk dengan label WR1, WR 2, WR3, WR4, WR5, WR6, WR7, WR8, WR9, WR10 dan WR11. Aktivitas enzim fungi pengurai serat yang mencakup lakase, peroksidase mangan dan lignin peroksidase, aviselase, carboksimetilselulase dan xilanase dianalisis dari fermentasi substrat padat. Data menunjukkan bahwa 5 fungi yang merupakan WR1, WR2, WR4, WR7 dan WR10 menghasilkan rasio tertinggi enzim pengurai lignin terhadap pengurai selulosa dan hemiselulosa. Sambungan apit fungi tersebut kemudian diamati di bawah mikroskop untuk menentukan filum. Keberadaan sambungan apit menunjukkan fungi termasuk filum Basidiomisetes.

Kata Kunci: Daun Kelapa Sawit, White Rot Fungi, Lignin, Enzim

ABSTRACT

Azmi MAB, Alias S, Azmi AFM, Ghani AAA, Shahudin MS, Goh YM, Noordin MM, Yusof MT, Zunita Z, Hassim HA. 2016. Determination of fibernolytic enzyme activities of white rot fungi isolated from oil palm fronds. JITV 21(2): 144-150. DOI: http://dx.doi.org/10.14334/jitv.v21i2.1362

Oil palm fronds (OPF) is widely used as the source of roughage for the farm animals. However, the full potential of OPF as animal feed is limited by their high lignin content which limits the rumen microbe's access to the cellulose and hemicellulose. White rot fungi (WRF) are a group of fungi belonging to basidiomycete phylum and are commonly found in decaying woody plant. They possess the ability to degrade lignin. This experiment aims to identify the phylum of the best lignin decaying fungi based on their ratio ligninolytic to cellulytic and hemicellulytic activities. In this experiment, 11 fungi species were isolated from decaying oil palm fronds. They are labelled as WR1, WR 2, WR3, WR4, WR5, WR6, WR7, WR8, WR9, WR10 and WR11. Their fibernolytic enzyme activities which include laccase, manganese peroxidase, lignin peroxidase, avicelase, carboxylmethylcellulase and xylanase are analysed from solid state fermentation method. It was found that 5 fungi species which are the WR1, WR2, WR4, WR7 and WR10 produced the highest ratio of lignin degrading enzyme to cellulose and hemicellulose degrading enzyme. The fungi are then analysed under microscope to determine the phylum of the fungi. From the observation, the fungi are identified to belong to the phylum of basidiomycetes due to presence of clamp connection.

Key Words: Oil Palm Fronds, White Rot Fungi, Lignin, Enzyme

INTRODUCTION

Malaysia is one of the largest palm oil producers. In 2008, Malaysia produced 17.7 million tonnes of palm oil based on 4,500,000 hectares used for the plantation of oil palm making it the second largest producer of palm oil in the world behind Indonesia (Abdullah & Sulaiman 2013). The large plantation of oil palm results in the large production of oil palm by-products. One of the main by-product of the palm oil plantation is the oil palm fronds. The oil palm fronds are obtained during pruning, felling and harvesting processes. Approximately 30,000,000 tonnes of oil palm fronds are produced in a year (Zahari & Farid 2011). Oil palm

fronds (OPF) has been widely used as animal feed in its various forms, those were freshly chopped, silage and pellets or cubes (Zahari & Farid 2011). Apart from that, the oil palm fronds are commonly used as a source of roughage. The usage of OPF as animal feed is limited due to its high lignin content and low protein content (Wan-Rosli et al. 2007). According to Zhang & Zhao (2010), the lignocellulosic biomass is made up from polymeric carbohydrates, which consist of cellulose, hemicellulose, and lignin. The digestibility of cellulose present in lignocellulosic biomass, such as OPF, is impeded by various physiochemical, structural, and compositional factors (Binod et al. 2012).The existence of hemicellulose and lignin in lignobiomass is aimed to impart strength to plant cell walls and protect cellulose from enzymatic degradation.

The white rot fungi consist of mostly basidiomycetes are efficient degrader of lignin (Smith & Thurnston 1997). The fungi are an excellent degrader of lignin via the excretion of extracellular enzymes. The enzymes are laccase, manganese peroxidase (MnP), and lignin peroxidase (Hassim et al. 2012). The usage of these enzymes to treat the oil palm fronds is believed to increase the efficiency of oil palm fronds by improving ruminal degradability.

The objective of this study is to determine the enzyme activity of white rot fungi isolated from decaying oil palm fronds and selecting 5 fungi species that are able to increase the degradability of OPF as animal feed. The fungi selected are then identified up to phylum level.

MATERIALS AND METHODS

Isolation and cultivation of white rot fungi

Sample of fungi ware isolated from rotten oil palm fronds collected from oil palm plantation located in Taman Pertanian Universiti, Universiti Putra Malaysia. The fungi were cultured on PDA agar plate at 30ºC for 7 days. Small portion of each fungus colonies were then transferred into fresh plate and cultured for another 7 days at 30ºC. The process was repeated until pure cultures were obtained. Plugs of 7 mm diameter from the purified cultures were then cultured on PDA agar plate for 5 days and the growth of the mycelia were observed (Namoolnoy et al. 2011).

Oil palm fronds fermentation

Solid state fermentation was carried out in 250-mL Erlenmeyer flask. Oil palm fronds collected from Ladang Kelapa Sawit, Taman Pertanian Universiti, Universiti Putra Malaysia, was divided and grinded into smaller pieces using warring blender. Each flask contains 15 g of grinded oil palm fronds. Each flask was added with 45 ml of Deionized water and 22.5 mg of glucose. The flasks were then autoclaved at 121ºC for 15 minutes. Three 10 mm diameter plugs from each isolated fungus were added to the sterile flask as inoculum. The flask was then covered with cotton ball and sealed with aluminium foil and incubated at room temperature (37ºC). The enzyme was extracted using 150 ml of deionized water added to each flask and placed inside rotary shaker for 3 hours. The mixture was then filtered. The filtrate was then centrifuged at 12,000 g at 4ºC and the supernatant was collected. The ~~step~~ sampling was repeated for 15 days, 30 days and 45 days fermentation for each isolated fungal species (Dinis et al. 2009).

Enzyme activity determination

Lignolytic enzyme

For screening of Laccase (EC 1.10.3.2) activity, a citrate-phosphate buffer was prepared by adding 1.921g to 100 ml of distilled water. The solution was labelled as solution A. Then, 3.5814 g of Na_2HPO_4 was added into 100 ml of distilled water. The solution was labelled as solution B. 50 ml of solution A was added to 50 ml of solution B and the pH is adjusted to pH 4.0. For the substrate, ABTS solution was prepared by adding 164.6 mg of ABTS to 10 ml of distilled water. The ABTS solution was then transferred to an Eppendorf tube. The mixture tube was prepared by adding 1300 µl citrate-phosphate buffer, 100 µl ABTS and 100 µl enzyme extract. For the blank, 1300 µl citrate-phosphate buffer was mixed with 100 µl ABTS. The mixtures were then covered with parafilm and were read at absorbance of 420 nm against blank (Dinis et al. 2009).

For screening of Lignin Peroxidase (EC 1.11.1.14; LiP), acid tartrate buffer was prepared by adding 3.752 g of the acid in 250 ml distilled water. The pH was then adjusted to 3.0 using acid/sodium tartrate buffer. The buffer was then kept in 4°C. The substrate was prepared by adding 0.105 ml veratryl alcohol with 25 ml distilled water. The solution was then store in 4°C in Eppendorf tubes. Hydrogen peroxide, H_2O_2 was then prepared according to manufacturer specification. The mixture tube was prepared by adding 2550 µl of acid tartrate buffer, 200 µl veratryl alcohol, 30 µl H_2O_2, and 200 µl enzyme extract. For the blank, the mixture solution was prepared with 2550 µl acid tartrate buffer, 200 µl veratryl alcohol and 30 µl H_2O_2. The tubes was read at absorbance of 238 nm against blank (Dinis et al. 2009).

For screening of Manganese Peroxidase (EC 1.11.1.13; MnP), tartrate buffer was prepared by adding 5.752 g sodium tartrate with 250 ml distilled water. The pH was adjusted to 5.0 by adding acid/sodium tartrate buffer. The solution was kept at 4°C. For the substrate, 0.25353 g of Manganese sulphate, $MnSO_4$ was mixed

with 50 ml of distilled water. It was then stored in 4°C. H_2O_2 was prepared according to manufacturer's standard. The mixture tube was prepared by adding 2550 µl sodium tartrate buffer, 200 µl manganese sulphate, 30 µl hydrogen peroxide, H_2O_2, and 200 µl enzyme extract. For the blank, the mixture is consist of 2550 µl sodium tartrate buffer, 200 µl manganese sulphate, and 30 µl hydrogen peroxide, H_2O_2. The absorbance was then read at 238 nm (Dinis et al. 2009).

Cellulolytic enzyme

For screening of Carboxylmethyl cellulase (CMcase) enzyme, the substrate was prepared by adding 1 g of carboxymethylcellulose to 100 ml distilled water. The solution was then stirred until it became homogeneous. The buffer used in this experiment is 0.1M citrate buffer at pH 4.8. The mixture is prepared by adding 1 ml of citrate buffer, 0.5 ml enzyme extract, and 0.5 ml carboxylmethyl cellulose. The tubes were then incubated at 39°C for 20 minutes. The reaction was stopped by adding 3 ml of dinitrosalicylic acid, DNS to each tube. The control tube was prepared by adding 1 ml of citrate buffer and 0.5 ml carboxylmethylcellulose, and 3 ml of DNS was added to all control tube. The reaction and control tubes were placed in boiling water bath for 10 minutes and were then read at absorbance of 575 nm. Calibration curve was made by plotting absorbance against glucose concentration. The glucose solution was prepared by dissolving 0.1 g of glucose in 100 ml of distilled water (Dinis et al. 2009).

For screening of Avicelase enzyme, the substrate was prepared by dissolving 1 g of Avicel microcrystalline in 100 ml of distilled water. The solution was then stirred until it became homogeneous. In this experiment, the buffer used was 0.1 M citrate buffer at pH 4.8. The mixture was prepared by adding 1 ml citrate buffer, 0.5 ml of enzyme extract and 0.5 ml Avicel cellulose microcrystalline solution. The tubes were then incubated at 39°C for 20 minutes. The reaction was stopped by adding 3 ml DNS solution to each test tube. Substrate control tube was prepared by adding 0.5 ml substrate with 1.5 ml citrate buffer. The enzyme control tube was prepared by mixing 0.5 ml enzyme extract with 1.5 ml citrate buffer. 3 ml of DNS was added to all control tubes. The reaction and control tubes were then placed inside boiling water bath for 10 minutes. The absorbance was then read at 575 nm against blank. Standard calibration curve was plotted against glucose concentration. The 0.1% glucose standard was prepared by dissolving 0.1 g glucose in 100 ml distilled water (Dinis et al. 2009).

Hemicellulolytic enzyme

Xylan was prepared by combining 0.25 g of xylan with 100 ml of distilled water. The mixture was then warmed at 70°C with continuous shaking. The assay mixture was prepared by adding 0.1M of citrate buffer at pH 4.8, 0.5 ml enzyme extract and 0.5 ml of 0.25% xylan solution. The tubes were incubated at 39°C for 30 minutes. The reaction was stopped by adding 3 ml of DNS to each reaction tube. The control tube for substrate was prepared by adding 0.5 ml 0.25% xylan with 1.5 ml citrate buffer. For the enzyme control tube, 0.5 ml of sample was mixed with 1.5 ml citrate buffer. 3 ml of DNS was added to all control tubes. All tubes were placed in boiling water bath for 10 minutes and absorbance was read at 575 nm against blank. Calibration curve was prepared by plotting absorbance against xylose concentration. The standard xylose solution was prepared by dissolving 0.1 g of xylose in 100 ml of distilled water (Dinis et al. 2009).

Fungi staining and phylum identification

Some fungi that showed the most desirable enzyme activity were selected. The selected fungi were then cultured on PDA agar plates. The plates were incubated at room temperature for 7 days. The grown fungi were stained with methylene blue and observed under light microscope. The presence of clamp connection in the hyphae were observed (Toda et al. 2012).

RESULTS AND DISCUSSION

All fungi recorded positive activity for the enzyme tested. In general, the fungi showed the highest lignolytic enzyme activity compared to other enzyme tested. All fungi recorded highest laccase activity on day 45 with exception of WR3. For the lignolytic enzyme activity which is presented in Figure 1, lignin peroxidase activity of fungi WR2 and WR6 recorded highest activity on day 15. WR1, WR4 and WR7 recorded highest activity on day 30. The rest of the fungi recorded the highest activity on day 45. For Manganese Peroxidase enzyme activity, WR2, WR4, WR5, WR7, and WR9 recorded the highest activity on day 15. Fungi WR1, WR6 and WR 11 recorded the highest Manganese Peroxidase on day 30. The rest of the fungi have the highest manganese peroxidase activity recorded on day 45.

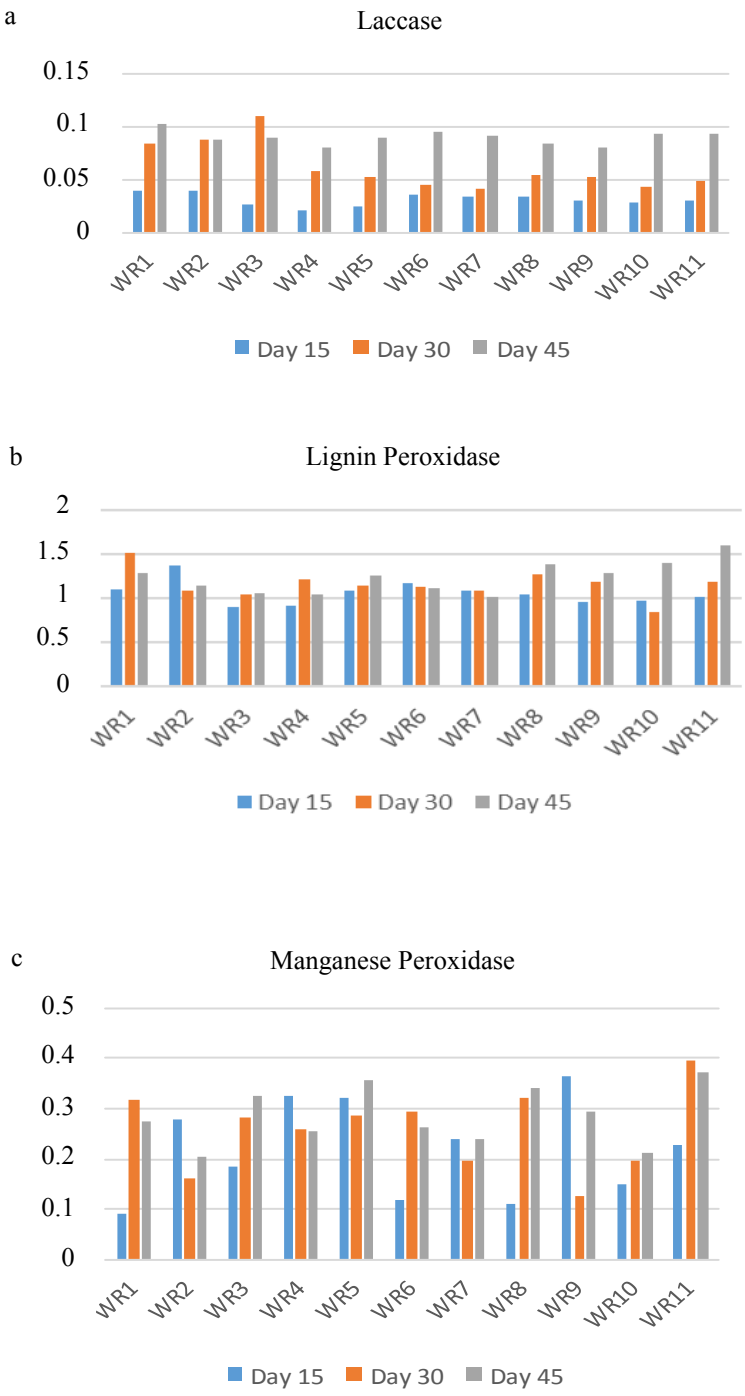


Figure 1. Activity of lignolytic enzymes: Laccase (a), lignin peroxidase (b), and manganese peroxidase (c) of isolated fungi after fermentation at oil palm fronds at 15, 30, and 45 days.

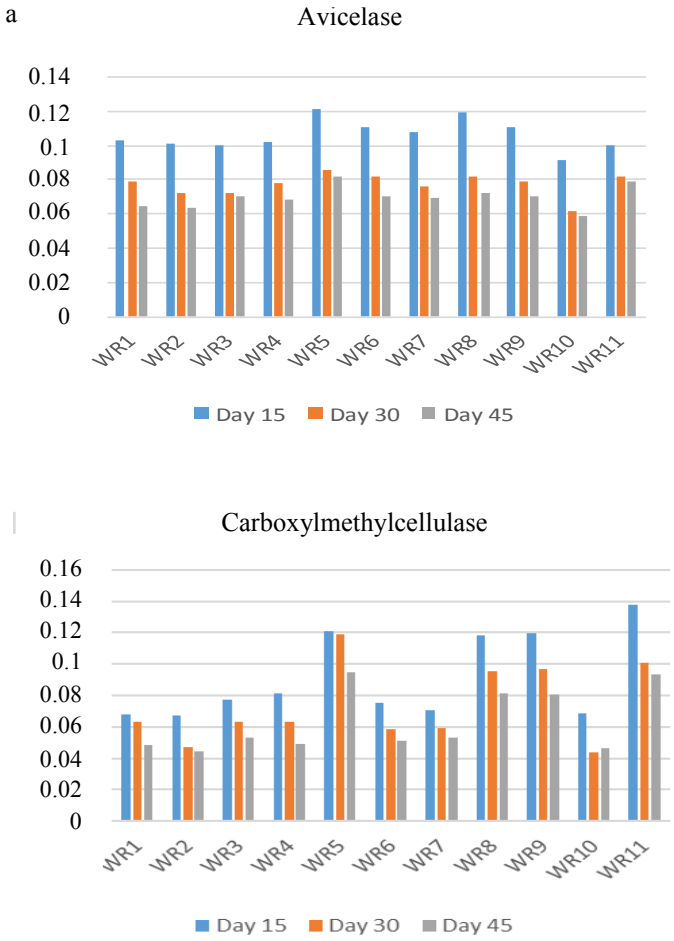


Figure 2. Activity of cellulolytic enzymes: Avicelase (a) and carboxylmethylcellulase (b) after fermentation at oil palm fronds at 15, 30, and 45 days.

The lignolytic enzyme activity is vital as it removes the lignin content which limits the access of rumen microbe toward the cellulose and hemicellulose. The presence of lignin also presents a problem as it is one of the main components of lignocellulosic biomass. The lignocellulosic biomass not only limits the physical access of rumen microbe toward the cellulose and hemicellulose, it also effectively inhibit the cellulose and hemicellulose from the reaction site via adsorption (Gusakov et al. 1985). Hence, it is important for the lignolytic enzyme to be high in selection criteria to increase the degradability of OPF.

In cellulolytic enzyme activity which is presented in Figure 2, for avicelase enzyme, the activity is highest at day 15 for all fungi followed by day 30 and 45 respectively. Carboxylmethylcellulase activity showed that WR5, WR 8, WR9, and WR11 had the highest average activity for all time period.

From the results, cellulose degrading enzymes show higher readings at 15 days and gradually decrease at 30 and 45 days. Alam et al. (2005) suggested that this is due to formation of aromatic water-soluble products from delignification process which suppress the cellulolytic action of the enzymes.

All fungi show low enzyme activity for xylanase with the exception of WR 5, WR8, WR9, and WR 11. However, the enzyme activity remains relatively constant for 15, 30 and 45 days observation with slight fluctuation. The activity of xylanase is the highest at 30 days fermentation period due to longer of fermentation time. However, nutrient depletion by the action of the fungi causes the xylanase activity to drop in 45 days fermentation time (Haq et al. 2002).

WR1, WR2 WR4, WR7, and WR10 have the optimal enzyme activity for pre-treating the OPF. The selection is based on the highest average recorded of lignin degrading enzyme as the lignin limits the

rumen's microbe accessibility to the cellulose and hemicellulose (Moore & Jung 2001; Dias et al. 2010; Azizi-Shotorkhoft et al. 2016). The selection criterium also considers the enzyme activity level for both cellulose and hemicellulose in which the levels need to be low. Hence, allowing more cellulose and hemicellulose to remain intact. This in turn allows rumen microbe to fully utilise the cellulose and the hemicellulose for the production of fatty acid (Hespell 1988). The differences between enzymes activity are not considered as the fungi were selected based on their average of total enzyme activity in the period of 15, 30, and 45 days for all enzyme tested. From previous research by Hassim et al. 2012, pre-treatment of OPF using white rot fungi shows increased in vitro ruminal degradability by 12%. The fungi inoculum will be used to pre-treat OPF with incubation period of 45 days.

For the identification of fungi phylum, the fungi selected, the WR1, WR2, WR4, WR7, and WR10 are identified as basidiomycetes. It is due to the presence of clamp connection in hyphae of the fungi. It is correlates to the ability of basidiomycetes as an excellent degrader of lignin (Hatakka 1994).

Further research is needed to further identify the species of the fungi Species determination will allow more understanding on their full enzyme potential to enable more efficient pre-treatment of OPF via fungal extracellular enzyme.

CONCLUSION

By measuring the activity of laccase, lignin peroxidase, manganese peroxidase, avicelase, xylanase and carboxylmethylcellulase, it can be concluded that WR1, WR2, WR4, WR7, and WR10 are the best fungi for pre-treatment of oil palm fronds as animal feed, more specifically, ruminants. The selected fungi are identified as basidiomycetes from the presence of the clamp connection in the hyphae.

REFERENCES

Abdullah N, Sulaiman F. 2013. The oil palm wastes in Malaysia. Biomass Now – Sustainable Growth and Use. 3:75-100. doi: 10.5772/55302.

Alam MZ, Muhammad N, Mahmat ME. 2005. Production of cellulase from oil palm biomass as substrates by solid state conversion. Am J Appl Sci. 2:569-572. doi: 10.3844/ajassp.2005.569.572.

Azizi-Shotorkhoft, Mohammadabadi T, Motamedi H, Chaji M, Fazaeli H. 2016. Isolation and identification of termite gut symbiotic bacteria with lignocellulose-degrading potential, and their effects on the nutritive value for ruminants of some by-products. J Anim Feed Sci Technol. 221:234-242. doi: 10.1016/j.anifeedsci. 2016.04.016.

Binod P, Satyanagalakshmi K, Sindhu R, Janu KU, Sukumaran RK, Pandey A. 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. Renew Energy. 37:109-116. doi: 10.1016/j.renene.2011.06.007.

Dias AA, Freitas GS, Marques GSM, Sampaio A, Fraga IS, Rodrigues MaM, Bezerra RMF. 2010. Enzymatic saccharification of biologically pre-treated wheat straw with white-rot fungi. Bioresource Technol. 101:6045-6050. doi: 10.1016/j.biortech.2010.02.110.

Dinis MJ, Bezerra RMF, Nunes F, Dias Aa, Guedes CV, Ferreira LMM, Rodrigues MaM. 2009. Modification of wheat straw lignin by solid state fermentation with white-rot fungi. Bioresour Technol. 100:4829-4835. doi: 10.1016/j.biortech.2009.04.036.

Gusakov aV, Sinitsyn aP, Klyosov aa. 1985. Kinetics of the enzymatic hydrolysis of cellulose: 1. A mathematical model for a batch reactor process. Enzyme Microb Technol. 7:346-352. doi: 10.1016/0141-0229(85)90114-0.

Hassim HA, Lourenço M, Goh YM, Baars JJP, Fievez V. 2012. Rumen degradation of oil palm fronds is improved through pre-digestion with white rot fungi but not through supplementation with yeast or enzymes. Can J Anim Sci. 92:79-87. doi: 10.4141/cjas2011-097.

Hatakka A. 1994. Lignin-modifying enzymes from selected white-rot fungi: Production and role from in lignin degradation. FEMS Microbiol Rev. 13:125-135. doi: 10.1111/j.1574-6976.1994.tb00039.x.

Haq, Khan IA, Butt WA, Ali S, Qadeer MA. 2002. Effect of carbon and nitrogen sources on Xylanase production by mutant strain of *Aspergillus niger* GCBMX-45. J Biol Sci. 2:143-144.

Hespell RB. 1988. Microbial digestion of hemicelluloses in the rumen. Microbiol Sci. 5:362-365.

Moore KJ, Jung HJG. 2001. Lignin and fiber digestion. J Range Manag. 54:420-430. doi: 10.2307/4003113.

Namoolnoy P, Phoolphundh S, Wongwicharn A. 2011. Biodegradation of lignin in oil palm fronds by white rot fungi. Kasetsart J Nat Sci. 45:254-259.

Smith M, Thurnston CF. 1997. Fungal laccases: Role in delignification and possible industrial application. In: Messerschmidt A, editor. Multi-Copper Oxidases. Singapore, New Jersey, London, Hong Kong: World Scientific. p. 253-259.

Toda T, Strausbaugh Ca, Rodriguez-Carres M, Cubeta Ma. 2012. Characterization of a Basidiomycete fungus from stored sugar beet roots. Mycologia. 104:70-78. doi: 10.3852/10-416.

Wan-Rosli WD, Law KN, Zainuddin Z, Asro R. 2007. Effect of pulping variables on the characteristics of oil-palm frond-fiber. Bioresour Technol. 93:233-240.

Zahari MW, Farid MM. 2011. Oil-palm by-products as feeds for livestock in Malaysia. p. 1-21. [accessed July 21th 2016]. http://umkeprints.umk.edu.my/1147/1/Paper%202.pdf.

Zhang Z, Zhao ZK. 2010. Microwave-assisted conversion of lignocellulosic biomass into furans in ionic liquid. Bioresour Technol. 101:1111-1114. doi: 10.1016/j.biortech.2009.09.010

AUTHOR GUIDELINES

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

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

AUTHOR GUIDANCE

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

SCRIPTWRITING SYSTEMATICS

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

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

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

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

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

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

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

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

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

Citation in the references:
Literatures in reference are written alphabetically based on the author's name. Same author is written sequentially starting from earlier order.

Example of reference writing

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

Book:

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

Proceeding:
Umiyasih U, Antari R. 2011. Penggunaan bungkil inti sawit dan kopra dalam pakan penguat sapi betina berbasis limbah singkong untuk pencapaian bobot badan estrus pertama >225 kg pada umur 15 bulan. Prasetyo LH, Damayanti R, Iskandar S, Herawati T, Priyanto D, Puastuti W, Anggraeni A, Tarigan S, Wardhana AH, Dharmayanti NLPI, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 192-199.

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

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

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

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

10. **Citation in text:**
Citation consists author's last name and publication year.

Example:

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

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

Dividing line is made in form of horizontal.

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

Write figure or graphic source under the title.

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

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

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

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

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

ETHICAL STATEMENT

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

Name :
Institution :
Title of Paper :

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

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

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

, 2016

Applicant,

_____

Author's colleague:

Name	Sign

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

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

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

COPYRIGHT TRANSFER FORM

Title of Paper :

Author :

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

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

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

, 2016

Approved by

Primary Author

Author's colleague:

Name	Sign

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

Acknowledgement

Editorial board and executive editor of Indonesian Journal for Animal and Veterinary Science (IJAVS) extent high appreciation to the expertises of peer reviewer of IJAVS (Volume 21 No. 2 2016).

1.	Prof. Dr. Budi Haryanto	:	Feed and Animal Nutrition - IRIAP
2.	Prof. Dr. drh. Agik Suprayogi, MVSc	:	Physiology and Pharmacology - Bogor Agricultural University
3.	Prof. Dr. Tjeppy D. Soedjana	:	Agricultural Economy - ICARD
4.	Dr. Nurhayati D. Purwantari	:	Cultivation - IRIAP
5.	Dr. Atien Priyanti	:	Agricultural Economy - ICARD

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

Jurnal Ilmu Ternak dan Veteriner

IJAVS Indonesian Journal of Animal and Veterinary Sciences

Volume 21, Number 2, June 2016 ISSN 0853-7380 E-ISSN 2252-696X

LIST OF CONTENT

Registered in: