ISSN 0853-7380
E-ISSN 2252-696X

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

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 23
Number 4
December 2018

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

JITV	Volume 23	Number 4	Page 159 - 222	Bogor, December 2018	ISSN 0853-7380

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

JITV	Volume 23	Number 4	Page 159-222	Bogor, December 2018	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 23 No 4, we proudly present articles from animal and veterinary sciences including genetics; reproduction and food technology. The articles published in this edition are: "Estimating breeding values for milk production and mastitis traits for Holstein cattle in Egypt"; "Genetic diversity and relationship among Bali cattle from several locations in Indonesia based on ETH10 Microsatellite Marker"; "Genotyping in the insulin-like growth factor 1 (IGF1/*SnaBI*) of Pasundan cattle with PCR-RFLP method"; "Serum biochemical, hormonal and fatty acid profiles during the late gestation of pregnancy ketosis in Boer cross goats"; "Improvement viability of *Lactobacillus casei* and *Bifidobacterium longum* with several encapsulating materials using extrusion method", and "Effect of electrical stimulation on physical and organoleptic properties of Muscovy duck meat".

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

Chief Editor

Bogor, December 2018

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 23, Number 4, December 2018 ISSN 0853-7380 E-ISSN 2252-696X

LIST OF CONTENT

Estimating Breeding Values for Milk Production and Mastitis Traits for Holstein Cattle in Egypt

Faid-Allah E

Department of Animal Production, Faculty of Agriculture, Minuofiya University, Egypt
E-mail: ifaidallah@yahoo.com

(received 01-08-2018; revised 17-12-2018; accepted 17-12-2018)

ABSTRAK

Faid-Allah E. 2018. Estimasi nilai breeding produksi susu dan sifat mastitis sapi Holstein di Mesir. JITV 23(4): 159-167. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1845

Penelitian ini dilakukan untuk mengevaluasi sapi pejantan dan induk betina secara genetis untuk sifat-sifat produksi susu dan mastitis pada 12 kelompok ternak sapi Holstein Mesir menggunakan Best Linear Unbiased Prediction melalui program MTDFREML. Data diperoleh dari sebuah peternakan komersial bernama Dena, yang terletak di Kairo-Alex Desert Road (80 Km), Menofia, Mesir. Data termasuk 4791 sapi betina, 4227 sapi induk dan 248 sapi pejantan yang mewakili periode 2007-2014. Estimasi nilai pemuliaan untuk sifat produksi susu yang berasal dari produksi susu kumulatif 90 hari (90-DM), produksi susu kumulatif selama 180 hari (180-DM), produksi susu kumulatif 270 hari (270-DM), produksi susu kumulatif pada 305 hari (305-DM), dan jumlah infeksi mastitis sekitar musim laktasi (MAST).Rata-rata dari 90-DM, 180-DM, 270-DM, 305-DM dan MAST adalah 3026,3±655,1 kg, 5873,3±1081,1 kg, 7891,1±2692,2 kg, 9611,2±1897,9 kg, dan 0,712±1,2 kali/paritas, secara berturut-turut. Estimasi heritabilitas untuk sifat tersebut adalah 0,11±0,016, 0,15±0,014, 0,18±0,012, 0,22±0,015 dan 0,09±0,029, secara berturut-turut; keragaman genetik 47206,2 kg, 175300,6 kg, 1304654,4 kg, 792411,6 kg dan 0,12 waktu/paritas, masing-masing; dan varians fenotipik adalah 429147.6 kg, 1168670.6 kg, 7248079.9 kg, 3601870.9 kg, dan 1.35 kali/paritas, secara beruturut-turut untuk masing-masing sifat. Nilai EBV sebagai rata-rata, SD, (Min: Max) untuk pejantan adalah 0,0±0,179 (-0,4: 0,66) untuk MAST, 0,0±86,176 (-263,1: 245,4) untuk 90-DM, 0,0±227,523 (-600,3: 800,3) untuk 180-DM, 0,0±413,48 (-323,3: 1277,7) untuk 270-DM dan 0,0±440,26 (-1280,9: 1565,1) untuk 305-DM. Juga, EBV untuk sapi induk adalah 0,0±0,055 (-0,14: 0,45) untuk MAST, 0,033±26,24 (-142,8: 103,0) untuk 90-DM, 0,074±76,81 (-360,2: 289,6) untuk 180-DM, -0,045±139.66 (-591.9: 529.2) untuk 270-DM dan 0.266±154.1 (-666.3: 617.6) untuk 305-DM. Hasil ini menunjukkan bahwa pemilihan sapi pejantan dan sapi induk akan meningkatkan sifat produksi dan mastitis susu dalam kawanan ini karena perbedaan yang luas dalam potensi genetik antara sapi pejantan dan sapi induk.

Kata Kunci: Heritabilitas, Nilai Pemuliaan, BLUP, Produksi Susu, Mastitis, Sapi Holstein

ABSTRACT

Faid-Allah E. 2018. Estimating breeding values for milk production and mastitis traits for Holstein cattle in Egypt. JITV 23(4): 159-167. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1845

This study was carried out to evaluate the sires and dams genetically for milk production and mastitis traits in Egyptian 12 herds of Holstein cattle using Best Linear Unbiased Prediction via MTDFREML program. The data was obtained from a commercial farm called Dena, located in Cairo-Alex Desert Road (80 Km), Menofia, Egypt. Data included 4791 cows, 4227 dams and 248 sires that represented the period from 2007 to 2014. Estimating breeding values for milk production traits as cumulative milk yield at 90 days (90-DM), cumulative milk yield at 180 days (180-DM), cumulative milk yield at 270 days (270-DM), cumulative milk yield at 305 days (305-DM), and number of mastitis infection around the season of lactation (MAST). The averages of the 90-DM, 180-DM, 270-DM, 305-DM and MAST were 3026.3±655.1 kg, 5873.3±1081.1 kg, 7891.1±2692.2 kg, 9611.2±1897.9 kg, and 0.712±1.2 time/parity, respectively. Estimates of heritability for the previous traits were 0.11±0.016, 0.15±0.014, 0.18±0.012, 0.22±0.015, and 0.09±0.029, respectively; genetic variance were 47206.2 kg, 175300.6 kg, 1304654.4 kg, 792411.6 kg and 0.12 time/parity, respectively; and phenotypic variance were 429147.6 kg, 1168670.6 kg, 7248079.9 kg, 3601870.9 kg, and 1.35 time/parity, respectively. The EBV values as average, SD, (Min: Max) for sires were 0.0±0.179 (-0.4: 0.66) for MAST, 0.0±86.176 (-263.1: 245.4) for 90-DM, 0.0±227.523 (-600.3: 800.3) for 180-DM, 0.0±413.48 (-323.3: 1277.7) for 270-DM and 0.0±440.26 (-1280.9: 1565.1) for 305-DM. Also, The EBVs for dams were 0.0±0.055 (-0.14: 0.45) for MAST, 0.033±26.24 (-142.8: 103.0) for 90-DM, 0.074±76.81 (-360.2: 289.6) for 180-DM, -0.045±139.66 (-591.9: 529.2) for 270-DM and 0.266±154.1 (-666.3: 617.6) for 305-DM. These results provide that the selection of sires and dams will improve the traits of milk production and mastitis in this herd because of the wide differences in genetic potential among sires and dams.

Key Words: Heritability, Breeding Value, BLUP, Milk Production, Mastitis, Holstein Cattle

INTRODUCTION

Egypt imports semen of foreign bulls via many companies around the world under the supervision of the Agricultural Ministry, Egypt. But, the final decisions were taken via farm managers in the order of selected bulls to inseminate the cow populations almost without real breeding consultancy. There is no real scientific genetic evaluation and follow up for these bulls and its effects of the genetic pool in cow populations in Egypt. There are many different trials of sire breeding value estimated by different researchers, under Egyptian conditions but almost not for application in the field. The estimating of BV's for sires for different economic traits (i.e., Milk production, milk quality, health, reproduction, durability, milking speed) is the main goal to successful breeding programs for cattle genetic improvement. One of the main criteria for enhancing the genetic potential of progenies in a herd is to use proven sires to transmit superior genetic potential for economic traits (Banik & Gandhi 2010). The sire evaluation based on milk yield was the most widely used criteria; milk yield is an important economic trait in livestock species. It represents a major source of income in most dairy enterprises (Al-Samarai et al. 2015). At the past decade, most dairy cattle breeding goals consist of functional traits and milk production traits (Miglior et al. 2005). In addition, functional traits are a collective term for all traits that increase efficiency by lowering the costs of production (Groen et al. 1997). Economic traits are generally controlled by genetic factors but environmental influences like, year of calving, the season of calving; parity and age at first calving have significant effects on milk yield (Pirzada 2011). Identification of the best sires with higher accuracy is of enormous importance for any breed improvement program, as sires are easily and rapidly spread in many herds used progeny test (Kumar & Chakravarty 2014). In the recent past, the best linear unbiased prediction (BLUP) procedure has been widely used as a standard method of sire evaluation.

The objective of this study was to estimate breeding values (EBVs) for Holstein sires and dams of milk production traits (90-DM, 180-DM, 270-DM, 305-DM), and MAST in 12 herds under Egyptian condition.

MATERIALS AND METHODS

Data and economic traits

Data were obtained from a commercial farm called Dena, located in Cairo-Alex desert road (80 Km), Menofia, Egypt. Data included 4791 cows as progenies of 4227 dams and 248 sires that represented the period from 2007 to 2014.

Random factors as sire, dam and fixed factors as farm (1-12), parity (1^{st} to $\geq 5^{th}$), year of calving (2007 to 2014), season of calving (summer 22/6 to 21/9, autumn 22/9 to 21/12, winter 22/12 to 21/3, spring 22/3 to 21/6) and AFC was used as co-variable. Sires having more than five progenies (on average 19 daughters per sire) were evaluated on the basis of Best Linear Unbiased Prediction (BLUP) method via MTDFREML program. Studied traits were milk production traits as cumulative milk yield at 90 days (90-DM), cumulative milk yield at 180 days (180-DM), cumulative milk yield at 270 days (270-DM), cumulative milk yield at 305 days (305-DM) milk yield and hygiene trait as number of mastitis infection around the season of lactation (MAST)

Animals were housed free in shaded open yards, grouped based to milk yield, and fed on TMR system around year based to NRC (NRC 2001). Holstein heifers were AI using semen of Holstein proven sires, around 350 kg of weight and pregnancy was detected at day sixty after service. The animals were machinery milked in milking parlour 3 times/day.

Genetic parameters and estimated breeding value

Genetic parameters and estimated breeding values (EBV) 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:

$$\mathbf{Y = Xb + Za + e}$$

Where:

Y	=	The vector of observations (milk production and hygiene traits)
b	=	Vector of fixed effects and covariates (farm, parity, calving year, calving season, covariate =afc)
a	=	Vector of random additive genetic direct effects (sire and dam)
X, Z	=	Known incidence matrices relating observations to the respective traits
e	=	Vector of residual effects (0,1)

RESULTS AND DISCUSSION

Descriptive statistics, heritability and genetic variance

Table (1) represents the mean, SD of studied traits for cumulative milk production traits as cumulative milk yield at 90 days (90-DM), cumulative milk yield at 180 days (180-DM), cumulative milk yield at 270 days (270-DM), cumulative milk yield at 305-days (305-DM) and number of mastitis infection around the season of lactation (MAST) are 3026.29±655.09 kg, 5873.31±1081.05 kg, 7891.1±2692.23 kg, 9611.18±1897.86 kg and 0.712±1.16 time/parity, respectively.

In Egypt, the mean of milk yield at 305 days for Holstein cows were 4295 kg (Ashmawy & Khalil 1990), 4736±1097 kg (Tawfik et al. 2000), 10847 kg (Abou-Bakr et al. 2006), 9038±1181 kg (Salem et al. 2006), 6118 kg (Abdel-Moez 2007), 8750 kg

Table 1. Descriptive statistics and genetic parameters of milk production traits and mastitis in Holstein cattle

Trait	Mean	SD	Genetic variance(σ^2G)	Phenotypic variance (σ^2p)	h^2	±se
MAST, time/parity	0.712	1.161	0.121	1.347	0.09	±0.029
90-DM, kg	3026.29	655.09	47206.23	429147.57	0.11	±0.016
180-DM, kg	5873.31	1081.05	175300.59	1168670.57	0.15	±0.014
270-DM, kg	7891.10	2692.23	1304654.37	7248079.85	0.18	±0.012
305-DM, kg	9611.18	1897.86	792411.59	3601870.88	0.22	±0.015

Records No = 4801
MAST = Number of mastitis infection around the season of lactation
90-DM = Cumulative milk yield at 90 days
180-DM = Cumulative milk yield at 180 days
270-DM = Cumulative milk yield at 270 days
305-DM = Cumulative milk yield at 305 days

(Ghoneim et al. 2011), 8455.4±1535.1 kg (Hammoud 2013), 8805±2024.3 kg (Rushdi et al. 2014) and 6384.95±1236.9 kg (Faid-Allah et al. 2016). In addition, the average of total milk yield for Friesian cattle in six commercial farms ranged from 3057 to 10900.7 kg (Farrag et al. 2017).

The mean value of the total milk yield in Holstein cows in Yemen was 3919.66±42.99 kg, this value ranged from 1720 to 5890 kg (Al-Samarai et al. 2015). In addition, the mean value of milk yield at 305 days for Ethiopian Holstein cows was 3,504.02±1,222.56 kg, this value ranged from 1,004 to 9,301 kg (Ayalew et al. 2017).In Friesian cattle, the average, SD of cumulative milk yield in 90, 150 and 180 day were 921±375, 1415±554, and 1637±637 kg (Khattab et al. 1993), 1837±512, 3392±744, and 3777±902 kg (Atil 1999) and 1475.49±527.49, 2337.08±860.78, and 2931.45±963.42 kg (Zein 2014), respectively.

The average, SD for cases number of clinical mastitis per lactation (time/parity) were 0.46±0.91 (Perez-Cabal et al. 2009), 1.99±1.62 (Zavadilová et al. 2015) and 0.38±0.4861 (Zavadilová et al. 2017).

Table (1) represents estimates of heritability (h^2±se) of studied traits for milk production traits as 90-DM, 180-DM, 270-DM, 305-DM, and MAST are 0.11±0.016, 0.15±0.014, 0.18±0.012, 0.22±0.015 and 0.09±0.029, respectively; genetic variance are 47206.23 kg, 175300.59 kg, 1304654.37 kg, 792411.59 kg and 0.12 time/parity, respectively; and phenotypic variance are 429147.57 kg, 1168670.57 kg, 7248079.85 kg, 3601870.88 kg, and 1.35 time/parity, respectively.

Estimates of h^2 are low to moderate and in agreement with most of the previous investigators. Heritability estimates for 305-day milk yield of Holstein were 0.17 (Meyer 1985), 0.13±0.040 (Abou-Bakr et al. 2006), 0.29 (Dadpasand et al. 2013), 0.24±0.12 (Endris et al. 2013), 0.20 (Kaygisiz 2013) and 0.25±0.001 (Rushdi et al. 2014).

In Egypt, heritability estimates of total milk yield for Friesian cows were 0.37 (El-Shalmani 2011), 0.14 (Shalaby et al. 2012) and 0.44 (Hammoud 2013) and 0.42 for 305-DM (Hammoud 2013).

The other researchers reported that heritability estimates ranged from 0.27 to 0.36 by method of REML in Holstein cattle (Tuna 2004; Atashi et al. 2006; Guler et al. 2010), and heritability estimates of milk yield for Brown Swiss in Turkey for first and all lactations were 0.23 and 0.19, respectively (Muammer et al. 2009). The present result was slightly lower than previously showed results for 305-DM and milk in the first three parities (0.51, 0.49, and 0.47) using the model of RR model for Holstein cattle in Netherland (De Roos et al. 2004). In spite of, it was still higher than 0.02 and 0.10 as heritability estimates of 305-DM and test day milk yields for Brown Swiss cattle in the USA, respectively (Suleyman & Ali 2008). The high estimate of heritability for total milk yield in Holstein cow in Yemen was 0.35±0.12 (Al-Samarai et al. 2015). However, the low estimate of heritability for 305 days milk yield from the first three lactation records was 0.15±0.04 in Ethiopian Holstein cattle (Ayalew et al. 2017).

Heritability estimates of cumulative milk yield in 90,150,180 days and 305 day milk yield were 0.213, 0.271, 0.286 and 0.327, respectively (Zein 2014); ranged from 0.22 to 0.52 for cumulative milk yield in 90 day (Khattab et al. 1993; Atil 1999; Khattab et al. 2000; Salem et al. 2000); ranged from 0.23 to 0.52 for cumulative milk yield in 150 day (Khattab et al. 1993; Atil 1999; Silvestre et al. 2005); ranged from 0.29 to 0.55 for cumulative milk yield in 90 day (Khattab et al. 1993; Atil 1999; Salem et al. 2000).

Estimates of heritability, genetic variance and phenotypic variance for 305 days milk yield (1807 records) in Holstein-Friesian cattle under Egyptian conditions were 0.149±0.045, 7275.0 kg, and 48757.0 kg; in addition, the previous estimates for total milk yield (1631 records) were 0.065±0.041, 4611.6 kg, and 70827 kg, respectively (Salem & Hammoud 2016). In addition, estimates of heritability, genetic variance and phenotypic variance for 305 days milk yield were

0.24±0.08, 81225 kg, and 1369720 kg in Holstein-Friesian cattle under Indonesian conditions (Malindo 2017).

Heritability estimates for mastitis trait were ranging from 0.09 to 0.11 in Norwegian Red cows (Heringstad et al. 2005), ranging from 0.07 to 0.15 (Zwald et al. 2006), 0.07±0.007 in Spanish Holstein cattle (Perez-Cabal et al. 2009), ranging from 0.11 to 0.13 for the number of mastitis cases per lactation (Wolf et al. 2010), ranging from 0.07:0.08 for clinical mastitis and ranging from 0.13 to 0.17 for subclinical mastitis in Swedish Holsteins (Urioste et al. 2012), ranging from 0.03 to 0.05 reported for clinical mastitis recorded from calving to 150 days after calving (Jamrozik et al. 2013), 0.04 for clinical Mastitis and 0.05 for number of cases of clinical mastitis in Spanish Holstein cattle (Pérez-Cabal & Charfeddine 2013), 0.09 (Zavadilová et al. 2015), 0.02 (Govignon-Gion et al. 2016), 0.05±0.007 (Zavadilová et al. 2017).

Differences in heritability estimates are caused by the correction for non-genetic factors, the records number used in estimation, the genetic constitution of the breed, research objects, management, climate and different model affecting genetic and environmental variances, and the estimation method (Abou-Bakr et al. 2006; Fu et al. 2017).

Estimated breeding value (EBV)

Estimated breeding value (EBV) is defined as the total genetic ability of an animal for a given trait. Thus, EBV refers to the value of an animal in a breeding program for a particular trait. In practice, breeders want to know the level of performance that can be expected from the offspring of certain individuals (Salem & Hammoud 2016). EBV is expressed as deviations from the population mean and sires were ranked based on their genetic merit (Faid-Allah et al. 2016).

Table (2) shows the EBV values as mean, SD, (Mini: Max) for MAST, 90-DM, 180-DM, 270-DM and 305-DM in sires and dams of Holstein cattle. The EBV values for sires were 0.0±0.179 (-0.4: 0.66) for MAST, 0.0±86.176 (-263.09: 245.43) for 90-DM, 0.0±227.523 (-600.32: 800.34) for 180-DM, 0.0±413.48 (-1323.29: 1277.72) for 270-DM and 0.0±440.26 (-1280.9: 1565.05) for 305-DM. The EBV values for dams were 0.0±0.055 (-0.14: 0.45) for MAST, 0.033±26.244 (-142.76: 103.02) for 90-DM, 0.074±76.811 (-360.21: 289.55) for 180-DM, -0.045±139.655 (-591.91: 529.21) for 270-DM and 0.266 ±154.1 (-666.31: 617.57) for 305-DM.

Table 2 and Figure 1 shows that the EBVs for sires had a wide range of all studied traits may be due to export sire from many sources around the world for Holstein cattle and change the desired goal of breeders from milk production traits as the main goal to functional and hygiene traits. In addition, The EBVs for dam had a smaller range than sires of all studied traits may be due to the selection of the superior cows inside the farms. Since 2012 the breeders were being used proven sires for mastitis and functional traits with lower breeding values for milk production traits, further improvement of mastitis performance and it explained the high genetic variation for sires EBVs.

In Egypt, EBVs estimated via Best Linear Unbiased Prediction (BLUP) method for 305-DM were in between –466 and 681 kg estimated from 1653 records of daughters/163 sires (Abdel-Glil 1991), and in between– 506 and 675 kg estimated from 1931 lactation records of daughters/76 sires (Atil & Khattab 1999). EBVs of all animals from 3464 records of Holstein cows/99 sires ranged from -4917.4 to 4731.3, -3863.1 to 3076.4, for total milk yield, 305-DM, respectively (Radwan et al. 2015). Predicted transmitting abilities for mastitis trait ranged from −0.0785 to 0.0965 for a number of cases of clinical mastitis, the ranges were from −0.2184 to 0.3884 (Perez-Cabal et al. 2009).

Table 2. Estimated breeding values for milk production and mastitis traits in Holstein cattle

	Traits	Min	Max	Mean	SD	Variance
Sire	MAST	-0.4	0.66	0.000	0.179	0.032
	90-DM	-263.09	245.43	0.000	86.176	7426.28
	180-DM	-600.32	800.34	0.000	227.523	51766.57
	270-DM	-1323.29	1277.72	0.000	413.48	170965.67
	305-DM	-1280.9	1565.05	0.000	440.26	193829.25
Dam	MAST	-0.14	0.45	0.000	0.055	0.003
	90-DM	-142.76	103.02	0.033	26.244	688.74
	180-DM	-360.21	289.55	0.074	76.81 1	5899.97
	270-DM	-591.91	529.21	-0.045	139.655	19503.58
	305-DM	-666.31	617.57	0.266	154.1	23746.68

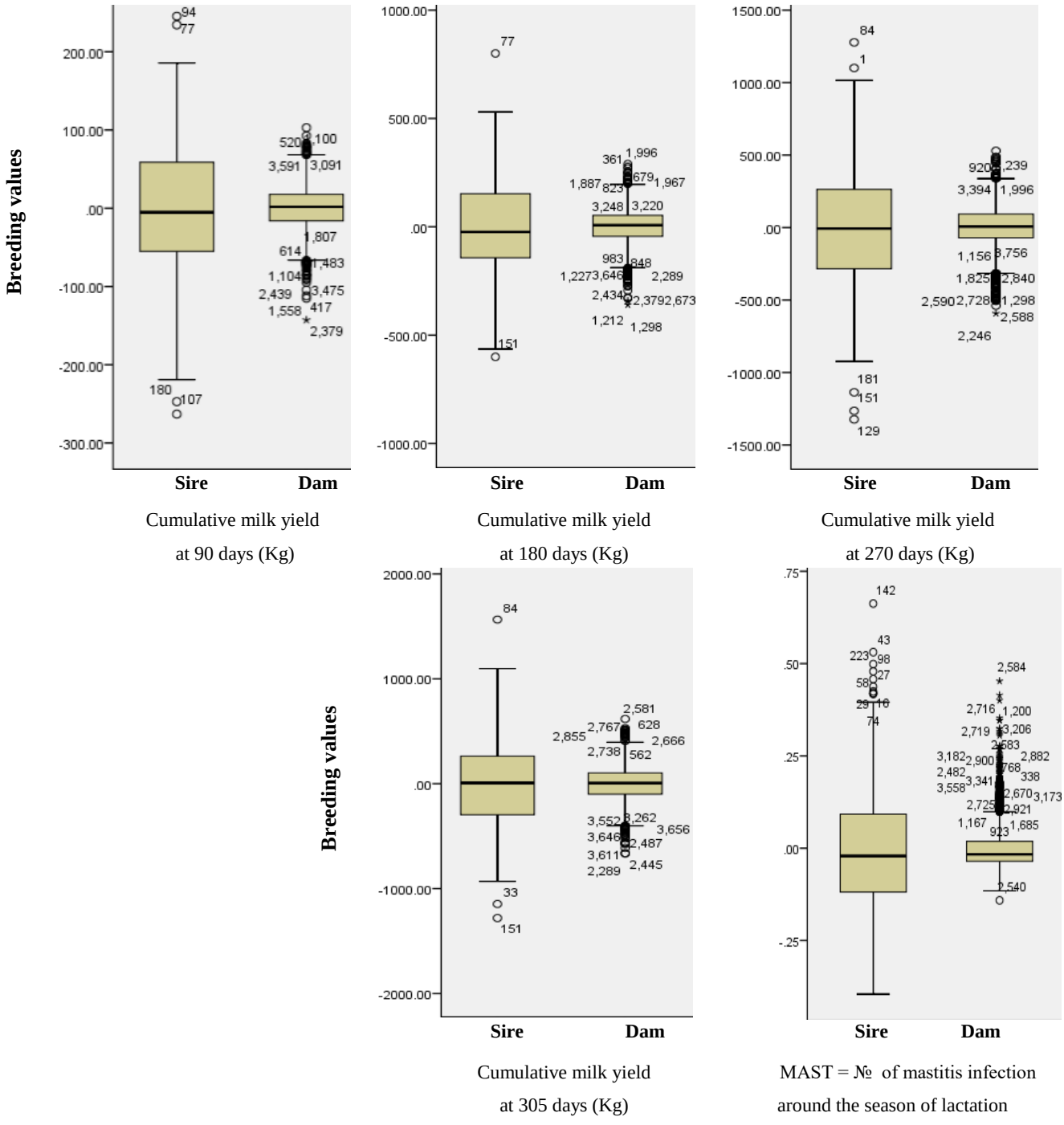


Figure 1. Box and Whisker plot of EBVs for milk production and mastitis traits in Holstein cattle.

EBVS for milk yield at first lactation using BLUP method ranged from 1262.90 to 1543.65 kg for Sahiwal sires (Duelkar & Kothekar 1999), as averageEBVs in Friesian cattle in Egypt as average (Min:Max) for 305-DM was 65.01 kg (-300.7: 706), total milk yield was 98.27 kg (-371.5:1105.7), cumulative milk yield in 90 days was 13.96 kg (-181:298), cumulative milk yield in 150 days was 15.73 kg (-362:381) and cumulative milk yield in 180 days was 49.70 kg (-397:390) (Zein 2014). EBVs for total milk yield , 305-DM from 3464 records of Holstein cows in Egypt ranged from -2096 to 2117, -372.9 to 315.9 kg, respectively, the values for dams were ranged from -1372 to 2113, -396.2 to 226.8 kg, respectively. EBVs for sires were ranged from -1095 to

1186, -245.9 to 171.9 kg for the previous traits, respectively (El-Bayoumi et al. 2015). EBVs of sires for the total milk yield (the average number of daughter/sire was 3.51) in between -471.88 and 443.8 with a marginal difference of 915.68 kg between lower and higher value in Holstein cattle in Yemen (Al-Samarai et al. 2015). EBVs for milk production in Holstein cattle in Egypt as a total milk yield ranged from -2736.6 to 3284.5, and for 305-DM ranged from -1698.0 to 1337.8 of cows, the EBVs for sires were ranged between -1056.8 to 659.1, and -737.1 to 621.9, respectively. EBVs for dams were ranged from -2835 to 2979.1, -985.2 to 1875.1 for the previous traits estimated from 1807 lactation records of daughters/73 sires, respectively (Salem & Hammoud 2016).

Table (3) shows that the values of EBV via MTDFREML program in percentiles of studied traits with great variation for 248 Holstein sires that imported for 12 herds in Egypt as semen for artificial insemination. High genetic variation was reported among the EBVs of sires as reported by many researchers in cattle (Dalal et al. 1999; Dubey et al. 2006; Banik & Gandhi 2006; Kumar et al. 2008; Moges et al. 2009).

Table (3) shows that the sires' EBVs ranged from -263.09 to 245.43 kg for milk yield at 90 days, -600.32 to 800.34 for milk yield at 180 days, -1323.29 to1277.72 for milk yield at 270 days, and -1280.9 to 1565.05 for milk yield at 305 days. Also, the sires' EBVs ranged from -0.4 to 0.66 for sire for the MAST.

About 50% out of 248 sires showed higher values than the average for milk production traits, 12 sires (5%) out of 248 sires as a best 5% showed higher EBVs than 148.61. 387.37. 720.35 and 813.57 kg for 90-DM, 180-DM, 270-DM, and 305-DM, respectively. About 50% out of 248 sires showed lower values than the average for the MAST. Also, 12 sires (5%) out of 248 sires showed lower EBVs than -.2747 for the MAST.

Milk yield EBVs ranged in -1013.9 to 1965.7 kg, The average of sires EBV via DFREML for milk yield at 1st parity was 3050.84 kg out of 57 sires 30 (52.63%) sires had EBV above the average and 24 (47.32%) sires had the below the average (Zutere 2008).

Table (4) shows that the dams' EBVs ranged from -142.76 to 103.02 for milk yield at 90 days, -360.21 to 289.55 for milk yield at 180 days, -591.91 to 529.21 for milk yield at 270 days, and -666.31 to 617.57 for milk yield at 305 days. Also, the dams' EBVs ranged from -0.14 to 0.45 for the dam for MAST

About 50% out of 4227 dams showed higher values than the average for milk production traits, 3170 dams (75%) out of 4227 dams as a best 75% showed higher EBVs than -16.0655, -44.1182, -70.4308 and -99.0659 kg for 90-DM, 180-DM, 270-DM, and 305-DM, respectively. About 50% out of 4227 dams showed lower values than the average for the MAST. Also, 3170 dams (75%) out of 4227 dams showed lower EBVs than-.0354for MAST.

The EBVS of milk yield at first lactation of Sahiwal sires using BLUP method ranged from 1153.95 to 2560.29 kg respectively. 38 out of 112 sires (33.93%) had EBVS above the average, while 74 sires (66.07%) had EBVS below the average. The top ranking sires had 35.46% genetic superiority over the overall average, whereas below average ranking sires had 38.95% low EBVS than the overall average (Singh & Singh 2016).

Table 3. EBVs Percentiles for studied Holstein sires

	EBVs Percentiles for 248 sires						
	5%	10%	25%	50%	75%	90%	95%
MAST	-.2747	-.2082	-.1200	-.0213	.0925	.2480	.3469
90-DM	-130.1630	-103.2672	-55.1935	-5.2273	58.7855	126.0289	148.6048
180-DM	-351.5393	-280.7055	-143.2290	-23.4620	153.2494	312.3312	387.3708
270-DM	-657.3163	-495.2965	-285.3595	-6.7086	264.6430	535.4776	720.3470
305-DM	-720.9358	-516.6709	-295.5986	6.8916	263.8430	612.9766	813.5737

Table 4. EBVs Percentiles for studied Holstein cows (as a dam)

	EBVs Percentiles for cows (as 4227 dam)						
	5%	10%	25%	50%	75%	90%	95%
MAST	-.0525	-.0458	-.0354	-.0164	.0185	.0709	.1129
90-DM	-45.6075	-34.5661	-16.0655	1.9182	17.8139	32.4053	42.1834
180-DM	-133.4583	-101.2163	-44.1182	7.3149	52.8633	95.7136	119.7531
270-DM	-251.4390	-180.8339	-70.4308	7.0513	93.1474	168.8614	221.1210
305-DM	-264.7312	-205.0363	-99.0659	5.9119	102.7117	188.5172	253.4017

EBVS for milk yield at first lactation using BLUP method ranged from 1262.90 to 1543.65 kg for Sahiwal sires (Duelkar & Kothekar 1999), as average was 1581.80 kg for Karan Fries sires (Kumar et al. 2008). BLUP via Derivative-free restricted maximum likelihood (DFREML) gave the EBV average ofSahiwal sires for 305-MY as 1503.99 kg and ranged from 1912.64 to 846.89. 49.38% out of 81 sires showed higher values than the average. In this method, 3 sires showed EBV over and above 20% as compared to the EBV average. Out of 81 sires, 7, 17 and 28 sires were having EBV s 15, 10 and 5% higher than the overall average (Banik & Gandhi 2010).

The EBVs of 51 Sahiwal sires (≥5 daughters/sire) were estimated from the 305-DM at 1st parity using four methods for sire evaluation. The mean of EBVs via BLUP "SAS", least squares "LSM", simple regressed least squares "SRLS" and Derivative-free restricted max likelihood "DFREML" methods were 1908.70 kg, 1869.91 kg, 1869.99 kg, and 1923.87 kg, respectively (Dongre & Gandhi 2014). EBVS for milk yield at first lactation using LSM as average were 1502.27 kg and it ranged from 830.41 kg to 2247.90 kg for Sahiwal cattle (Banik & Gandhi 2006).

CONCLUSION

The genetic evaluation was carried by MTDFREML method for milk production and mastitis traits using 4791 records on daughters of 4227 dams and 248 sires of Holstein cattle. The great variations were detected in EBVs for Holstein sires in milk production and mastitis traits. The high importance of genetic evaluation for Holstein sires and dams in our herds to help the breeder for making a decision in a breeding program. Re-evaluate Holstein sires under Egyptian conditions as a Bio-Safety protocol. Since 2012 the farm is being used proven sires for mastitis and functional traits with lower breeding values for milk production traits, further improvement of mastitis performance and it explained the high genetic variation for sires' EBVs.

ACKNOWLEDGEMENT

The cooperation of Dena for Agriculture Investments; that provided data is kindly acknowledged. Dena farms' breed of choice is Holstein imported from the USA. It relies on artificial insemination by top global semen providers to maintain the genetic potential of the breed.

REFERENCES

Abdel-Glil M. 1991. Sire differences for milk production traits in Friesian cattle (Thesis). [Ash Sharqiyah (Egypt)]: Zagazig University.

Abdel-Moez K. 2007. A genetic study on lactation traits of Holstein cattle and its crosses with European Friesian in a commercial farm in Egypt (Thesis). [Cairo (Egypt)]: Cairo University.

Abou-Bakr S, Alhammad HOA, Sadek RR, Nigm AA. 2006. Productive and reproductive characteristics of Holstein cows raised under intensive farming system in Egypt. Egyptian J Anim Prod. 43:91-98.

Al-Samarai F, Abdulrahman Y, Mohammed F, Al-Zaidi F, Al-Anbari N. 2015. Comparison of several methods of sire's evaluation for total milk yield in a herd of Holstein cows in Yemen. Open Vet. J. 5:11-17.

Ashmawy A, Khalil M. 1990. Single and multi-trait selection for lactation in Holstein-Friesian cows Egypt. J Anim Prod. 272:171-184.

Atashi H, Moradi S, Abdolmohammadi A. 2006. Study of Suggested Measures of Milk Yield Persistency and Their Relationships. Int J Agric Bio. 8:387-390.

Atil H. 1999. Ratio and regression factors for prediction 305-day production from part lactation milk records in a herd of Holstein Friesian cattle. Pak J Biol Sci. 2:31.

Atil H, Khattab A. 1999. Seasonal age correction factors for 305-day milk yield in Holstein cattle. Pak J Bio Sci. 2:296-300.

Ayalew W, Aliy M, Negussie E. 2017. Estimation of genetic parameters of the productive and reproductive traits in Ethiopian Holstein using multi-trait models. Asian-Australasian J Anim Sci. 30:1550-1556.

Banik S, Gandhi R. 2006. Animal model versus conventional models of sire evaluation in Sahiwal cattle. Asian-Australasian J Anim Sci. 19:1225-1228.

Banik S, Gandhi R. 2010. Sire evaluation using single and multiple trait animal models in Sahiwal cattle. Indian J Anim Sci. 80:269-270.

Boldman K, Kriese L, Van Vleck L, Van Tassell C, Kachman S. 1995. A manual for use of [MTDFREML] A Set of programs to obtain estimates of variances and covariances. Washington DC (USA): USDA/AES.

Dadpasand M, Zamiri M, Atashi H. 2013. Genetic correlation of average somatic cell score at different stages of lactation with milk yield and composition in Holstein cows. Iranian J Vet Res. 14:190-196.

Dalal D, Rathi S, Raheja K. 1999. Relationship between sires estimated breeding values for first lactation and lifetime traits in Hariana cattle. Indian J Anim Sci. 72:398-01.

De Roos A, Harbers A, de Jong G. 2004. Random herd curves in a test-day model for milk, fat, and protein production of dairy cattle in the Netherlands. J Dairy Sci. 87:2693-2701.

Dongre V, Gandhi R. 2014. Study on sire evaluation methods in Sahiwal cattle. Indian J Vet Anim Sci Res. 43:174-179.

Dubey P, Singh C, Prasad R. 2006. Relationship between sire's estimated breeding values for first lactation and lifetime traits and ranking of sires in Sahiwal and its cross. Indian J Anim Sci. 76:824-828.

Duelkar P, Kothekar M. 1999. Sire evaluation considering FLY for improvement of lifetime production in Sahiwal. Indian J Anim Sci. 69:240-242.

El-Bayoumi K, El-Tarabany M, Abdel-Hamid T, Mikaeil O. 2015. Heritability, genetic correlation and breeding value for some productive and reproductive traits in Holstein cows. Res Opin Anim Vet Sci. 5:65-70.

El-Shalmani A. 2011. Evaluation of production performance in relation to genetic structure of some economical traits in Friesian cows (Thesis). [Alexandria (Egypt)]: Alexandria University.

Endris M, Tumwasorn S, Sopannarath P, Prasanpanich S. 2013. Genotype by region interaction on milk production traits of Holstein crossbred dairy cows in Thailand. Kasetsaart J Nat Sci. 47:228-237.

Faid-Allah E, Ghoneim E, Ibrahim A. 2016. Estimated variance components and breeding values for pre-weaning growth criteria in Romney sheep. JITV. 21:73-82.

Farrag F, Shalaby N, Gabr A, El Ashry M. 2017. Evaluation of Friesian cattle performance at first lactation under different Egyptian conditions. J Anim Poultry Prod Mansoura Univ. 8:7-11.

Fu XF, Lu LL, Huang X, Wang Y, Tian K, Xu X, Fang J, Cheng L, Guo Z, Tian Y. 2017. Estimation of genetic parameters for 305 days milk yields and calving interval in Xinjiang Brown cattle. Agric Sci. 8:46-55.

Ghoneim E, Abd-Ellatif M, Abdelharith H, Abd Elhamid M. 2011. Genetic parameters for age at first calving and lifetime milk yield traits in Friesian cattle in Egypt. Minufiya J Agric Res. 36:913-923.

Govignon-Gion A, Dassonneville R, Baloche G, Ducrocq V. 2016. Multiple trait genetic evaluation of clinical mastitis in three dairy cattle breeds. Animal. 10:558-565.

Groen A, Steine T, Colleau J, Pedersen J, Pribyl J, Reinsch N. 1997. Economic values in dairy cattle breeding, with special reference to functional traits. EAAP working group. Livest Prod Sci. 49:1-21.

Guler O, Yanar M, Akbulut O. 2010. Variance component estimation for heritability of Gamma lactation curve traits of Holstein Friesian cattle. The Indian Vet J. 87: 35-38.

Hammoud M. 2013. Genetic aspects of some first lactation traits of Holstein cows in Egypt. Alex J Agric Res. 58:295-300.

Heringstad B, Chang Y, Gianola D, Klemetsdal G. 2005. Genetic analysis of clinical mastitis, milk fever, ketosis, and retained placenta in three lactations of Norwegian Red cows. J Dairy Sci. 88:3273–3281.

Jamrozik K, Koeck A, Miglior F. 2013. Genetic and genomic evaluation of mastitis resistance in Canada. Interbull Bulletin. 47:23-26.

Kaygisiz A. 2013 Estimation of genetic parameters and breeding values for dairy cattle using test-day milk yield records. J Anim Plant Sci. 23:345-349.

Khattab A, El Ariain M, Atil H. 2000. Estimation of milk producing ability of Holstein Friesian cattle in a commercial herd in Egypt. Pak J Biol Sci. 3:1076.

Khattab A, Sultan Z, Kassab M, Salem A. 1993. Inheritance of part lactation records in Friesian cows in Egypt. J Agric Res Tanta Univ. 19:747.

Kumar A, Gandhi R, Singh A, Haile A. 2008. Comparison of animal model with other conventional methods of sire evaluation for milk production in Karan Fries cattle. Indian J Anim Sci. 78:1393-1396.

Kumar V, Chakravarty A. 2014. Genetic study on performance of Murrah buffalo bulls. Int J Develop Res. 4:1482-1484.

Malindo R. 2017. Genetic and non-genetic analysis of milk production and reproduction traits in Holstein-Friesian cattle reared at BBPTU-HPT Baturraden Central Java. (Thesis). [Bogor (Indones)]: Bogor Agricultural University.

Meyer K. 1985. Genetic parameters for dairy production of Australian Black and White cows. Livest Prod Sci. 12:205-219.

Miglior F, Muir BL, Van Doormaal BJ. 2005. Selection indices in Holstein cattle of various countries. J Dairy Sci. 88:1255-1263.

Moges T, Singh C, Barwal R, Kumar D, Singh C. 2009. Evaluation of sires using different multitrait sire evaluation methods in crossbred cattle. Indian J Dairy Sci. 62:1-4.

Muammer T, Mehmet C, Mehmet S. 2009. Genetic parameters of 305-day milk yield for Brown Swiss reared in the Bahri Dağdaş International Agricultural Research Institute in Turkey. Kafkas Univ Vet Fak Derg. 15:397-400.

[NRC] National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th ed. Washington DC (USA): National Academy of Science, National Research Council.

Pérez-Cabal M, Charfeddine N. 2013. Genetic relationship between clinical mastitis and several traits of interest in Spanish Holstein dairy cattle. Interbull Bulletin. Nantes. France. 47:77-81.

Perez-Cabal M, de los Campos G, Vazquez A, Gianola D, Rosa G, Weigel K, Alenda R. 2009. Genetic evaluation of susceptibility to clinical mastitis in Spanish Holstein cows. J Dairy Sci. 92:3472–3480.

Pirzada R. 2011. Estimation of genetic parameters and variance components of milk traits in Holstein-Friesian and British-Holstein Dairy cows. Kafkas Univ Vet Fak Derg. 17:463-467.

Radwan H, Abo Elfadl E, Fardos A. 2015. Estimates of population parameters for some economic traits in Holstein Friesian cows by using statistical program. Global Vet. 14:129-135.

Rushdi H, Ibrahim M, Shaddad N, Nigm A. 2014. Estimation of genetic parameters for milk production traits in a herd of Holstein Friesian cattle in Egypt. J Anim Poult Prod Mansoura Univ. 5:267-278.

Salem A, Kassab M, Khattab A, Hussien A. 2000. Age correction factors for milk yield traits in a commercial herd of Friesian cattle in Egypt J Agric Sci Mansoura Univ. 25:3945.

Salem M, Esmail H, Sadek R, Nigm A. 2006. Phenotypic and genetic parameters of milk production and reproductive performance of Holstein cattle under the intensive production system in Egypt. Egypt J Anim Prod. 43:1-10.

Salem M, Hammoud M. 2016. Estimates of heritability, repeatability and breeding value of some performance traits of Holstein cows in Egypt using repeatability animal model. Egyptian J Anim Prod. 53:147-152.

Shalaby N, El-Barbary A, Oudah E, Helmy M. 2012. Genetic parameters and breeding values of some productive and reproductive traits Friesian cattle in Egypt. Proceeding the 15th AAAP Animal Science Congress 26-30. Thummassat Univ. Rangift, Thailand.

Silvestre A, Petim-Batista F, Colaco J. 2005. enetic parameter estimates of Portuguese dairy cows for milk, fat and protein using a Spline Test-Day Model. J Dairy Sci. 88:1225.

Singh J, Singh C. 2016. Evaluation of sires using different sire evaluation methods on the basis of first lactation traits in Sahiwal cattle. J Vet Sci Technol. 7:296.

Suleyman C, Ali K. 2008. Breeding value estimation of dairy cattle using test day milk yields for Brown Swiss cows reared at Ulaş State farm. J Anim Vet Adv. 7:703-706.

Tawfik E, Mohsen M, AY S, EL-Awady H. 2000. Study on Friesian Herds Raised in Egypt and Germany (I. Estimate of non-genetic effects and genetic parameters). Arch Tierz Dummerstorf. 43:101-114.

Tuna Y. 2004. Studies on the genetic constitution of Black and White dairy cattle raised in Tahirova State farm. Pak J Bio Sci. 7:931-933.

Urioste J, Franzén J, Windig J, Strandberg E. 2012. Genetic relationships among mastitis and alternative somatic cell count traits in the first 3 lactations of Swedish Holsteins. J Dairy Sci. 95:3428–3434.

Wolf J, Wolfova M, Stipkova M. 2010. A model for the genetic evaluation of number of clinical mastitis cases per lactation in Czech Holsteins cows. J Dairy Sci. 93:1193-1204.

Zavadilová L, Štípková M, Šebková N, Svitáková A. 2015. Genetic analysis of clinical mastitis data for Holstein cattle in the Czech Republic Arch. Anim Breed. 58:199-204.

Zavadilová L, Štípková M, Svitáková A, Krupová Z, Kašná E. 2017. Genetic parameters for clinical mastitis, fertility and somatic cell score in Czech Holstein cattle. Ann Anim Sci. 17:1007-1018.

Zein D. 2014. Genetic relationships among some dairy traits in Friesian cattle in Egypt (Thesis). [Al Minufya (Egypt)]: Menoufya University.

Zutere R. 2008. Estimates of breeding values for dairy cattle using test-day milk yields. Latvian J Agronomy. 10:293-299.

Zwald N, Weigel K, Chang Y, Welper R, Clay J. 2006. Genetic analysis of clinical mastitis data from onfarm management software using threshold models. J Dairy Sci. 89:330-336.

.

Genetic Diversity and Relationship among Bali Cattle from Several Locations in Indonesia Based on ETH10 Microsatellite Marker

Margawati ET, Volkandari SD, Indriawati, Ridwan M

Research Center for Biotechnology, Indonesian Institute of Science, Jalan Raya Bogor Km.46, Cibinong, Bogor 16911
E-mail: endangtri@hotmail.com

(received 12-10-2018; revised 08-12-2018; accepted 10-12-2018)

ABSTRAK

Margawati ET, Volkandari SD, Indriawati, Ridwan M. 2018. Keragaman genetik dan hubungan genetik diantara sapi Bali dari beberapa lokasi di Indonesia berdasarkan Marker Mikrosatelit ETH10. JITV 23(4): 168-173. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1915

Sapi Bali adalah salah satu sapi lokal Indonesia yang sampai saat ini mengindikasikan adanya *inbreeding*. Tujuan dari penelitian ini adalah untuk menganalisis keragaman genetik dan hubungannya diantara sapi Bali dari beberapa lokasi di Indonesia berdasarkan *marker* mikrosatelit ETH10. Sebanyak 94 sampel DNA yang terdiri dari 89 sapi Bali dan 5 Banteng dilakukan analisis. Sapi Bali berasal dari enam (6) lokasi di Indonesia yaitu Pulukan Bali (15), Nusa Penida Bali (15), Bima Nusa Tenggara Barat (14), Mataram (10), Riau (20), dan Kalimantan Selatan (15) sedangkan Banteng berasal dari Prigen Malang sebanyak 5 sampel. Marker mikrosatelit ETH10 dilabel HEX untuk digunakan dalam amplifikasi. Alel dianalisis menggunakan software Cervus 3.0.7 dan GenAlex 6. Hasil menunjukkan bahwa terdapat lima (5) alel pada marker ETH10 yaitu 209, 213, 215, 217, dan 219 pb. Rata-rata nilai heterosigositas teramati (Ho) dan harapan (He) adalah 0,46±0,05 dan 0,60±0,03. Lima dari enam lokasi sapi Bali telah terindikasi *inbreeding* kecuali sapi Bali dari Mataram. Jarak terjauh kekerabatan genetik yaitu antara sapi Bali dari Mataram dan Riau, sedangkan jarak terdekat yaitu sapi Bali dari Kalimantan Selatan dengan Mataram. Banteng sangat dekat dengan sapi Bali dari Nusa Penida dan berjarak sangat jauh dengan sapi Bali dari Kalimantan Selatan. Riset ini mengindikasikan terdapat *inbreeding* pada sapi Bali, karenanya diperlukan perhatian pada rotasi pejatan dan penyebaran semen untuk peningkatan performa sapi Bali.

Kata Kunci: Sapi Bali, Mikrosatelit ETH10, Keragaman Genetik, Hubungan Genetik

ABSTRACT

Margawati ET, Volkandari SD, Indriawati, Ridwan M. 2018. Genetic diversity and relationship among Bali cattle from several locations in Indonesia based on ETH10 Microsatellite Marker. JITV 23(4): 168-173. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1915

Bali cattle is one of local beef cattle in Indonesia, up to present its performance indicated an inbreeding occurrence. This study was aimed to analyze the genetic diversity and relationship among Bali cattle from several locations in Indonesia based on ETH10 microsatellite marker. Ninety-four (94) DNA samples (89 Bali cattle; 5 Banteng) were analyzed. The Bali cattle samples were from 6 locations in Indonesia (15 Pulukan; 15 Nusa Penida; 14 Bima West Nusa Tenggara/WNT; 10 Mataram, WNT; 20 Riau; 15 South Borneo). DNA Banteng samples were collected from Prigen Malang of East Java. Microsatellite marker of ETH10 labelled HEX was used for amplification. Alleles were analyzed by using Cervus 3.0.7 and GenAlex 6.5. Result showed that there were five (5) alleles found in ETH10 marker *i.e.,* 209; 213; 215; 217; and 219 bp. Average of observed (Ho) and expected (He) heterozygosity were 0.46±0.05 and 0.60±0.03, respectively. Five (5) out of 6 locations were in breeding occurrence except Bali cattle from Mataram was not inbreeding. The longest genetic relationship was between Bali cattle from Mataram and Riau whereas the closest distance was Bali cattle from South Borneo with Mataram. Banteng was closest to Bali cattle from Nusa Penida and the longest was to Bali cattle from South Borneo. This finding indicates there is inbreeding in Bali cattle, therefore it needs to be concerned in bull rotation and semen distribution for increasing the Bali cattle performance.

Key Words: Bali Cattle, ETH10 Microsatellite, Genetic Diversity, Genetic Relationship

INTRODUCTION

Bali cattle is one of beef cattle gene pools in South East Asia and as one of local beef cattle in Indonesia (Margawati et al. 2015a). Bali cattle has been spread almost throughout the archipelago. Decreasing of performance (quantitative trait) and abnormal body color pattern is a sign of inbreeding occurrence. Abnormal pattern of body color occurred in several locations of Indonesia *i.e.*, Kupang, Nusa Tenggara Timur (NTT) (Tabun et al. 2013), Lombok, Nusa Tenggara Barat (NTB) (Sudrana et al. 2014), South Borneo (Lindell 2013), and Taro Gianyar, Bali island (Margawati et al. 2015b). The signs of abnormal color in Bali cattle are white spotted color, black color for cow, reddish body color for bull, reddish for soak leg color, and albino (Hardjosubroto & Astuti 1993).

Molecular approach technology could be used to analyze of genetic variation by using mitochondrial DNA and applying of microsatellite analysis. Investigation of that matter is very important for future monitoring of gene flow in populations, conservation and determining of the level of inbreeding within and between breeds (Hetzel & Drinkwater 1992). Microsatelitte marker is one of the most powerful means for studying the genetic diversity, calculation of genetic distance, detection of bottlenecks and admixture because of high degree of polymorphism (Sharma et al. 2015). (FAO 2011) recommended 30 microsatelitte markers for cattle to analyze genetic diversity and ETH10 is one of those microsatelittes. The range of ETH10 alleles is approximately 207-231 bp.

Utilization of marker microsatellite analysis could be used to investigate the level of inbreeding of Bali cattle in several locations. The objective of this study was to analyzed the genetic diversity and genetic relationship of Bali cattle from several locations in Indonesia based on ETH10 microsatelitte marker.

MATERIALS AND METHODS

Blood samples

A total of ninety-four (94) blood samples (89 samples of Bali cattle and 5 samples of Banteng) was collected (Table 1), from different areaas as presented in Figure 1. Fresh blood samples in 3 ml were taken from *vena jugularis* and collected into a vacuntainer containing anticoagulant (K_3EDTA).

Table 1. Samples of Bali cattle and Banteng in this study

Samples	Origin	N
Bali cattle	Pulukan, Bali	15
Bali cattle	Nusa Penida ,Bali	15
Bali cattle	Mataram, West Nusa Tenggara/WNT	10
Bali cattle	Bima, WNT	14
Bali cattle	South Borneo	15
Bali cattle	Riau	20
Banteng	Safari Garden2, Prigen, Malang	5
Total		94

DNA extraction and quantification

Genomic DNA was extracted using a High salt method (Montgomery & Sise 1990) for blood samples while DNA from tail hair was extracted using gSYNCDNA extraction kit (Geneaid). The quality of DNA samples were measured using a spectrophotometer (GeneQuant Pro, Amersham UK) to check DNA concentration and purity. DNA samples were prepared at 50 ng/µl.

Amplification of ETH10 microsatellite marker

Polymerase Chain Reaction (PCR) method was used for amplification of ETH10 marker. A pair primer of ETH10 marker was F: 5'-GTTCAGG ACTGGCCCTGCTAACA-3'; R: 5'-CCTCC AGCCCACTTTCTCTTCTC-3' and labelled with HEX fluorescent as recommended by (FAO 2011). A total volume of PCR reaction was 20 µl consisting of PCR master mix (K-2012, Bioneer) and mixed with17 µl free nuclease water, 1 µl primer *Forward* dan *Reverse* (10 pmol/µl), and 1 µl DNA template (50 ng/µl). The PCR reaction was run using a Thermal cycler (Eppendorft, Germany) which set up as initial denaturation at 95°C for 1 minute, followed by 30 cycles of 95°C for 1 minute (denaturation), 58°C for 1 minute (annealing) and 72°C for 1 minute (extension) then final extension at 72°C for 5 minutes (Sharma et al. 2015). The PCR product was checked using agarose gel 2% and visualized under UV light (MUV21, MajorScience, USA). The PCR products were then sent to sequence services (First BASE) for reading of DNA target fragment.

Fragment DNA analysis and phylogenetic tree construction

DNA fragment of ETH10 locus was calculated to analyze the genetic diversity. Parameters of this study were allele frequency, observed number of allele (N), expected number of allele (Ne), observed (Ho) and expected (He) Heterozygosity, Hardy Weinberg Equilibrium (HWE), Wright's *F*- statistics (Fis, F_{ST}, F_{IT}), and gene flow. Cervus 3.0.7 (Kalinowski et al. 2007) and GenAlex 6.5 (Peakall & Smouse 2012) software were used to estimate basic population of genetic descriptive statistic for each population. Phylogenetic tree was constructed by MEGA 5.0 software (Tamura et al. 2011) with a Neighborjoining (1,000 boostraps) method.

RESULTS AND DISCUSSION

Genetic diversity

Genetic diversity of Bali cattle and Banteng from several locations was established using an ETH10 microsatellite marker. Five variants of alleles were found *i.e.*, 209; 213; 215; 217; and 219 bp. According to (FAO 2011), allele ranges of ETH10 marker are 207-231 bp. Variation and number alleles of each of populations and locations are presented in Table 1. Distribution of alleles in this study was showed in Figure 2. Alleles of 213 and 217 bp were dominant alleles in all locations. (FAO 2007) has specified a minimum of four different alleles per locus for

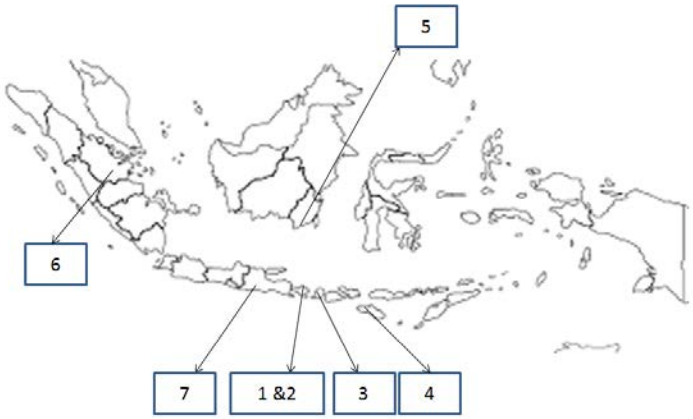


Figure 1. Location of samples collection. 1: Pulukan Bali; 2: Nusa Penida Bali; 3: Mataram WNT; 4: Bima WNT; 5: South Borneo; 6: Riau and 7: Prigen Malang East Java.

Table 1. Number of alleles in each population of Bali cattle and Banteng

Breed	Origin	Number of alleles	Variation of allele (bp)
Bali	Pulukan, Bali	3	209; 213; 217
Bali	Nusa Penida, Bali	5	209; 213; 125; 217; 219
Bali	Riau	4	209; 213; 217; 219
Bali	South Borneo	3	209; 213; 217; 219
Bali	Mataram, WNT	2	213; 217
Bali	Bima, WNT	3	209; 213; 217
Banteng	Prigen, Malang	3	213; 217; 219

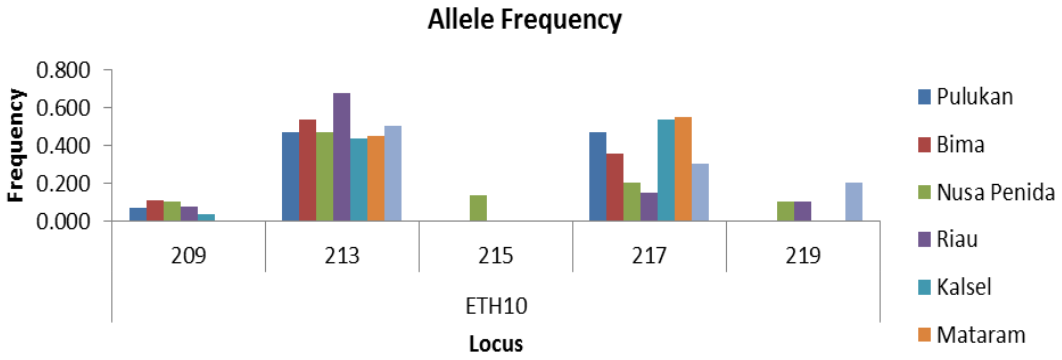


Figure 2. Distribution of alleles based on ETH10 locus.

evaluation of genetic differences between breeds. Using the (FAO 2007) criterion, therefore, polymorphic was only detected in Bali cattle from Nusa Penida (5 alleles) and Riau (4 alleles). Previous study of (Sharma et al. 2015) reported that the number of alleles in Indian breeds cattle was found 14 alleles with allele range 185-221 bp in ETH10 locus. Furthermore, (Cervini et al. 2006) found that Nellore cattle has 8 alleles (205-219 bp), whereas four alleles on ETH10 locus has been found in Sudanese cattle (Hussein et al. 2015) and five alleles in Buśa cattle from Bosnia (Rogic et al. 2011).

The observed number of alleles (Na) was 2 in Bali cattle from Mataram WNT to 5 in Bali cattle from Nusa Penida Bali and the average alleles of populations was 3.29±0.36 (Table 2). While the expected number of alleles (Ne) varied from 1.980 (Mataram, WNT) to 3.383 (Nusa Penida, Bali). The result showed a lower expected number of alleles (Ne) compared to the observed number of alleles (Na) (Na>Ne) in all of Bali cattle from all locations.

Table 2. Genetic diversity of Bali cattle and Banteng

Breed	Origin	N	Na	Ne	Ho	He	Fis
Bali	Pulukan, Bali	15	3	2.273	0.400	0.579	0.286
Bali	Nusa Penida, Bali	15	5	3.383	0.467	0.704	0.338
Bali	Riau	20	4	2.025	0.500	0.506	0.012
Bali	South Borneo	15	3	2.113	0.200	0.527	0.620
Bali	Mataram, WNT	10	2	1.980	0.500	0.495	-0.010
Bali	Bima, WNT	14	3	2.347	0.571	0.574	0.004
Banteng	Prigen, Malang	5	3	2.632	0.600	0.620	0.032
Mean±SD		13.43±188	3.29±0.36	2.40±0.19	0.46±0.05	0.60±0.03	0.18±0.10

N= number of individuals; Na= observed number of alleles; Ne= expected number of alleles; Ho= observed heterozygosity; He= expected Heterozygosity; Fis= inbreeding coefficient

Table 3. PIC value and global F-statistics of ETH10 locus

Locus	PIC	Fis	Fit	Fst	Nm
ETH10	0.533	0.188	0.237	0.060	3.896

PIC= Polymorphic Information Content, Fis= coeficient of inbreeding, Fit= deviation of Hardy Weinberg proportion in total population, Fst= Wright's standardized variance, Nm= gene flow

Table 4. Pairwise population matrix of Nei genetic distance

Pulukan	Bima	Nusa Penida	Riau	South Borneo	Mataram	Banteng	
0.000							Pulukan
0.021	0.000						Bima WNT
0.126	0.066	0.000					Nusa Penida
0.178	0.080	0.052	0.000				Riau
0.007	0.052	0.181	0.254	0.000			South Borneo
0.010	0.058	0.188	**0.257**	**0.001**	0.000		Mataram WNT
0.091	0.070	**0.067**	0.073	**0.119**	0.116	0.000	Banteng

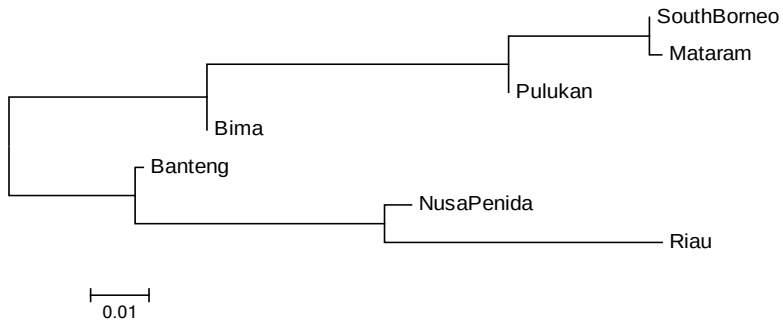


Figure 3. Genetic relationship of Bali cattle and Banteng from several locations.

Heterozygosity value is a suitable used to measure the genetic diversity within populations (Hanslik et al. 2000). The estimates of observed (Ho) and expected (He) heterozygosity of Bali cattle from all locations and populations were 0.46±0.05 and 0.60±0.03, respectively. The calculation showed that Ho value was lower than He value. The highest Ho value was found

in Banteng population whereas the lowest Ho value was found in Bali cattle from South Borneo.

In this study, polymorphic information content (PIC) showed a considerable high variation (0.533). (Botstein et al. 1980) classified PIC into three groups, ie those are low (PIC<0.25), moderate (0.25<PIC<0.50) and high (PIC>0.50) variation. Based on Wright's *F*- statistics analysis, Fis value detected that populations were in random (Sodhi et al. 2008). Fit value is greater than Fst value (Fit>Fst), therefore Fis value would be positive. It interpreted that there was inbreeding occurrence (Weir 1996); Sharma et al. 2015). Average of Fis value in this study was positive (0.188) (Table 3). Fis value in all populations and locations were mostly positive. The negative value was only found in Bali cattle from Mataram WNT (Table 2). According to (Nei 1987) Fis>0 showed deficiency of heterozygotes and excess of homozygotes. It could be influenced by a number of factors *i.e*., assertive mating, presence of population substructure within the populations (Wahlud effect) or null alleles.

The number of migrants per generation (gene flow or Nm) was observed to be 3.896% (Table 3) which perhaps was not influenced by genetic structure in all of populations. It seems due to of using similar bull for matings in quite long time (Sharma et al. 2015) reported that Nm value in Indian cattle reached 5.608%.

Genetic relationship

Based on Pairwise population matrix of Nei genetic distance analysis (Table 4) within population, the longest genetic distance was investigated between Bali cattle from Mataram WNT (0.257) and Riau whereas the closest genetic distance was between Bali cattle from South Borneo (0.001) with Bali cattle from Mataram WNT. Banteng represented as indigenous cattle of Indonesia. Banteng or *Bos javanicus* is known as a wild cattle (Syed-Shabtar et al. 2013) and Bali cattle is domesticated cattle from Banteng 3,500 BC (Mohamad et al. 2009). Findings of this present study showed that the closest genetic distance with Banteng was Bali cattle from Nusa Penida Bali and the longest genetic distance was Bali cattle from South Borneo. This result was proved by Phylogenetic tree analysis (Figure 3) which found that Banteng was at the same group to that Bali cattle from Nusa Penida and Riau but was in different group to that Bali cattle from South Borneo, Pulukan Bali, Mataram WNT and Bima WNT. It might be related with the trade market and distribution of Bali cattle among islands in Indonesia. (The Agency of Livestock and Animal Health of NTB Province 2014) reported that demand of Bali cattle breeding stock originated from West Nusa Tenggara reached 14,651 heads and 1,718 out of 14,651 heads was transported to South Borneo.

This recent study proved that inbreeding already occurred in Bali cattle population from several locations in Indonesia, it was also happened in Banteng from Safari Garden 2 Prigen Malang of East Java. Rotation of bulls or semen distribution (for artificial insemination needs) for mating is important to be properly managed. Those managements are necessary and very important to avoid inbreeding occurrence in Bali cattle population throughout the country.

CONCLUSION

The genetic diversity and relasionship was detected in the study of on ETH10 microsatelitte marker with five-variant alleles. Polymorphic was found in two locations of Bali cattle population *i.e* Nusa Penida Bali and Riau. The occurence of inbreeding detected in Bali cattle from Pulukan Bali, Nusa Penida Bali, Mataram WNT, Bima WNT, and Riau and Banteng from Prigen 2 Malang of East Java. The longest genetic relationship was found between Bali cattle from Mataram WNT and Riau while the closest distance was Bali cattle from South Borneo to Mataram WNT. The closest genetic distance to Banteng was showed by Bali cattle from Nusa Penida Bali and the longest genetic distance was Bali cattle from South Borneo.

ACKNOWLEDGEMENT

This study was supported by DIPA TEMATIK 2017 LIPI. The authors thank to Evi Alfiah Taukhid for excellent assistance in the laboratory. Appreciation was also delivered to staff of Safari Garden2 Prigen Malang who allowed us to collect tail hair of Banteng.

REFERENCES

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 32:314-331.

Cervini M, Henrique-Silva F, Mortari N, Matheucci Jr E. 2006. Genetic variability of 10 microsatellite markers in the characterization of Brazilian Nellore cattle (*Bos indicus*). Genet Mol Biol. 490:486-490.

[FAO] Food and Agriculture Organization. 2007. The state of the world's animal genetic resources forfood and agriculture. In brief. Richskowsky DP, editor. Rome (Ital): Food and Agriculture Organization.

[FAO] Food and Agriculture Organization. 2011. Molecular genetic characterization of animal genetic resources. Rome (Ital): Food and Agriculture Organization.

Hanslik S, Harr B, Brem G, Schlotter C. 2000. Microsatellite analysis reveals substantial genetics differentiation between contemporary new wold and old world Holstein-Friesian populations. Anim Genet. 31:31-38.

Hardjosubroto W, Astuti J. 1993. Buku pintar peternakan. Jakarta (Indones): PT. Gramedia Widiasarana.

Hetzel D, Drinkwater R. 1992. The Use of DNA technologies for the conservation and improvement of animal genetic resources. Rome (Ital): FAO Expert Consultation on the Management of Global Animal Genetic Resources.

Hussein IH, Alam SS, Makkawi AAA, Abdoon AS, Hassanane MS, Abdoon AS, Hassanane MS, Commons C. 2015. Genetic diversity between and within Sudanese Zebu cattle breeds using microsatellite markers. Res Genet. 2015:135483.

Kalinowski S, Taper M, Marshall T. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternityassigment. Mol Ecol. 16:1099-1106.

Lindell IC. 2013. Phenotyping of Bali cattle and interviewing farmers in Indonesia - a minor field study. Uppsala (Swedia): Swedish University of Agriculture Sciences.

Margawati E, Indriawati, Volkandari S, Ridwan M. 2015a. Identification of pure breed cattle by using molecular approach. Putra RARS, Widya RAE, Insani GA, Noviandi CT, Amrullah AHK, editors. Proceeding 6th International Seminar on Tropical Animal Production. Yogyakarta (Indones): Gadjah Mada University. p. 426-431.

Margawati E, Indriawati, Volkandari S, Ridwan M. 2015b. Penyusunan arsitektur genetik sapi Bali dengan pendekatan kuantitatif dan analisis DNA. Laporan Akhir DIPA TEMATIK 2015. Pusat Penelitian Bioteknologi LIPI.

Mohamad K, Olsson M, Tol HTA Van, Mikko S, Vlamings BH, Rodrı H, Colenbrander B, Lenstra JA. 2009. The origin of Indonesian cattle and conservation genetics of the Bali cattle breed. Reprod Domes Anim. 47:18-20.

Montgomery GW, Sise JA. 1990. Extraction of DNA from sheep white blood cells. New Zeal J Agr Res. 33:437-441.

Nei M. 1987. Molecular evolutionary genetics. New York (USA): Columbia University Press.

Peakall R, Smouse P. 2012. GenAlEx 6.5: Genetic analysis in Excel, population genetic software for teaching and research update. Bioinformatic. 28:2537-2539.

Rogic B, Tomic L, Vazic B, Jelic M, Jovanovic S, Savic M. 2011. Assessment of genetic diveristy of Busa cattle from Bosnia and Herzegovina using Microsatellite DNA marker. Arch Biol Sci. 63:1077-1085.

Sharma R, Kishore A, Mukesh M, Ahlawat S, Maitra A, Pandey AK. 2015. Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers. BMC Genet. 16:73.

Sodhi M, Mukesh M, Ahlawat S, Sobti R, Gohlot G, Mehta S, Prakash B, Mishra B. 2008. Genetic diversity and structure of two prominent Zebu cattle breeds adapted to the Arid Region of India inferred from microsatellite polymorphism. Biochem Genet. 46:124-136.

Sudrana IP, Lestari, Jan R, Rozy T, Kasip L. 2014. Pola warna menyimpang pada sapi Bali di Kabupaten Lombok Barat. Jurnal Penelitian UNRAM. 18:54-63.

Syed-Shabtar S, Rosli M, Mohd-Zin N, Romaino S, Fazly-Ann Z, Mahani M, Abas-Mazni O, Zainuddin R, Yaakop S, Md-Zain B. 2013. The molecular phylogenetic signature of Bali cattle revealed by maternal and paternal markers. Mol Biol Reprod. 40:5165–5176.

Tabun AC, Hartatik T, Sumadi. 2013. Identification of Melanocortin 1 Receptor (MC1R) gene based on coat color of Bali cows of Kupang By using the PCR-RFLP method. J Indones Trop Anim Agric. 38:86–91.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular Evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 28:2731-2739.

The Agency of Livestock and Animal Health of NTB Province. 2014. Livestock Statistic of West Nusa Tenggara. NTB (Indones): The Agency of Livestock and Animal Health of NTB Province.

Weir BS. 1996. Genetic Data Analysis II Methods for Discrete Population Genetic Data. Massachusetts (USA): Sinauer Associaes, Inc Publisher.

Genotyping in the Insulin-like Growth Factor 1 (IGF1/*Sna*BI) Gene of Pasundan Cattle with PCR-RFLP Method

Putra WPB[1], Nugraheni ST[2], Irnidayanti Y[2], Said S[1]

[1]*Research Center for Biotechnology - Indonesian Institute of Sciences, Indonesia*
[2]*Department of Biology, Faculty of Life Sciences - Jakarta State University, Indonesia*
E-mail: widya.putra.lipi@gmail.com

(received 13-09-2018; revised 08-12-2018; accepted 08-12-2018)

ABSTRAK

Putra WPB, Nugraheni ST, Irnidayanti Y, Said S. 2018. Genotyping pada gen Insulin-like Growth Factor 1 (IGF1/*Sna*BI) sapi Pasundan dengan metode PCR-RFLP. JITV 23(4): 174-179. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1862

Gen *Insulin-like Growth Factor* 1 (IGF1) pada mamalia berfungsi untuk mengontrol pertumbuhan tulang dan otot. Oleh karena itu gen IGF1 banyak digunakan sebagai salah satu kandidat gen untuk seleksi ternak. Penelitian ini bertujuan untuk mengidentifikasi genotip gen IGF1 (ekson 1) menggunakan metode PCR-RFLP dengan enzim restriksi *Sna*BI (TAC*GTA). Sebanyak 90 ekor sampel DNA sapi Pasundan dari Kabupaten Ciamis dan Pangandaran, Jawa Barat telah digunakan pada penelitian ini. Hasil penelitian ini menunjukkan bahwa seluruh sampel yang dianalisis memiliki genotip CC dengan alel C sebagai alel yang umum pada gen IGF1/*Sna*BI. Genotip CC yang diperoleh pada penelitan ini disebabkan karena terdapat mutasi transisi pada posisi basa ke g.218T/C (GenBank: KF202095). Mutasi ini menyebabkan perubahan asam amino dari *methionine* (AUG) menjadi *valine* (GUG). Disimpulkan bahwa gen IGF1/*Sna*BI pada sapi Pasundan bersifat monomorfis dan tidak dapat digunakan untuk seleksi molekuler.

Kata Kunci: Gen IGF1/*Sna*BI, PCR-RFLP, Monomorfis, Mutasi, Sapi Pasundan

ABSTRACT

Putra WPB, Nugraheni ST, Irnidayanti Y, Said S. 2018. Genotyping in the Insulin-like Growth Factor 1 (IGF1/*Sna*BI) gene of Pasundan cattle with PCR-RFLP method. JITV 23(4): 174-179. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1862

The Insulin-like Growth Factor 1 (IGF1) gene is important to control sceleton and muscle development. Therefore, IGF1 gene was widely used as the candidate gene for livestock selection. This research was carried out to identify the genotype of IGF1 gene (exon 1) using PCR-RFLP method with *Sna*BI restriction enzyme (TAC*GTA). Total of 90 DNA samples of Pasundan cows from Ciamis and Pangandaran Regencies were used in the present study. Research reveals that all sample in the animal studied have CC genotype with C allele as the common allele in IGF1/*Sna*BI gene. The CC genotype that obtained in the present study was conducted by the transition mutation position g.218T/C (GenBank: KF202095). This mutation was changed the amino acid from methionine (AUG) to valine (GUG). It was concluded that IGF1/*Sna*B1 gene of Pasundan cattle is monomorphic and can not used for molecular selection.

Key Words: IGF1/*Sna*B1 Gene, , Monomorphic, Mutation, Pasundan Cattle, PCR-RFLP

INTRODUCTION

Pasundan cattle is one of Indonesian native cattle that adapt well in West Java province of Indonesia. This cattle was declared as native cattle of Indonesia through decision Ministry of Agriculture of Republic Indonesia No: 1051/Kpts/SR.120/10/2014. Indrijani et al. (2012) reported that the Pasundan cattle originates from crossbreeding batween Bali cattle (*Bos javanicus*) and Ongole or Madura cattle (*Bos indicus*) since a hundred years ago. The Pasundan cattle was kept by farmers in West Java as beef cattle. As the native beef cattle in Indonesia, the genetic improvement of Pasundan is important to increase productivity. Recently, the genetic improvement of Pasundan cattle can be obtained through molecular selection using some candidate genes that affecting the productivity.

Insulin-like Growth Factor 1 (IGF1) or somatomedin gene is one of the candidate that widely used as molecular selection in cattle (Szewczuk 2016). The bovine IGF1 is a small secreted peptide with 70-90 amino acids and molecular weight about 7500 kb and located on chromosome 5 with 6 exons and 5 introns (Miller et al. 1991; Rose 2002; Mullen et al. 2011). The IGF1 was produced in various body tissues, especially in the liver is produced mainly by influenced growth hormone. The IGF1 is a mediator of many biological effects, for example, it increases the absorbtion of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes,increases the synthesis of lipids, stimulates the production of progesterone in granular cells and intervenes in the synthesis (Etherton 2004). Hence, the

IGF1 gene is known to play an important role in various aspects of muscle growth and development.

One single nucleotide polymorphism (SNP) of g.218T/C was detected in the bovine IGF1 gene and can be detected with PCR-RFLP method with *Sna*BI restriction enzyme (Ge et al. 2001). Since year 2000, this SNP was identified in the 5'UTR of bovine IGF1 gene (GenBank: AH009378.2) but in year 2013 this SNP was confirmed in the exon 1 (GenBank: KF202095). Polymorphism in IGF1/*Sna*BI was affected to the productivity traits of cattle i.e. body weight at three months in Hanwoo cattle (Chung & Kim 2005), serum concentration of IGF1 at 14 days post partum in Friesian Holstein (FH) cattle (Mirzaei et al. 2012), service per conception and days open in FH cattle (Ararouti et al. 2013), breeding value of milk and fat yields in Iranian Holstein bulls (Mehmannavaz et al. 2010), growth traits in beef cattle (Siadkowska et al. 2006; Reyna et al. 2010; Szewczuk et al. 2013), carcass traits (Reyna et al. 2010) and meat colour in FH bulls (Ardicli et al. 2018). This research was aimed to detect SNP in the exon 1 of IGF1/*Sna*BI gene in Pasundan cattle using PCR-RFLP method. The result of this study is important to find the candidate gene for productivity traits of Pasundan cattle in the future.

MATERIALS AND METHODS

Animals and bloods sample

Total of 90 DNA sample of Pasundan cows from Ciamis (57 heads) and Pangandaran (33 heads) Regencies were used in this study. The blood samples (3-5 ml) were taken from cocygeal vein using venoject tube and collected in vaccutainer tubes containing anticoagulant (K_2EDTA). The DNA extraction was obtained with Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan) following the producers instruction. The extracted DNA was appropriately recorded and stored at -20°C for next analysis.

Amplification and genotyping of IGF1/*Sna*BI gene

The DNA fragments of IGF1 gene in Pasundan cattle were successfully to amplify by primer forward: 5'- ATTACAAAGCTGCCTGCCCC -3' and reverse: 5'-ACCTTACCCGTATGAAAGGAATATACGT -3' (Ge et al. 2001). This primer was amplified of IGF1 gene fragments along 246 according to GenBank: KF202095 (Figure 1). The DNA samples for amplification were prepared by additing 0.60 µl ddH_2O; 4.0 µl DNA solution (10 - 20 ng) and 0.20 µl of primers (1 pmol) into tube of 5.0 µl of PCR kit Green Taq (Thermo Scientific, USA). The amplification of DNA fragments were performed by using mastercycler gradient machine (Eppendorf, Germany). The PCR machine was programmed for initial denaturation 94°C for 4 min., and followed by 36 cycles of denaturation at 94°C at 45 sec., annealing at 57°C for 45 sec., elongation at 72°C for 1 min. and final elongation at 72°C for 10 min. Genotyping of IGF1 gene was performed by RFLP analysis. The RFLP was performed with 4.2 µl of PCR product that consisted of 4.45 µl of ddH_2O; 0.25 µl of *Sna*BI restriction enzyme (TAC*GTA); 1.0 µl buffer 10x and 0.1 µl acetylated BSA. The mixture was incubated in waterbath at 37°C for 2 h and visualized using 2% agarose gel with GelRed staining (Biotium, USA) and captured in GBOX Documentation System (Syngene, UK).

Data analysis

Data analysis of the IGF1 gene were consisted of allele frequency, genotype frequency, expected heterozygosity (H_e), observed heterozygosity (H_o), number of effective allele (n_e), polymorphic informative content (PIC) and Chi-square value (χ^2) value based on Nei & Kumar (2000).

RESULTS AND DISCUSSION

The IGF1 gene fragment was successfully amplified along 246 bp (Figure 2). The RFLP analysis showed that the IGF1/*Sna*B1 of Pasundan cattle was monomorphic with CC genotype as the common genotype (Table 1). The SNP of g.218T/C in the IGF1 gene can be detected with *Sna*BI restriction enzyme. All cattle in the present study are homozygote CC animals and signed by one fragments along 246 bp on the agarose gel. The n_e value in SNP of g.218T/C was 1.00 and indicated that only one common allele that found in this SNP. The PIC value in the IGF1/*Sna*B1 gene was 0.00 and indicated that this gene can not be used for molecular selection. In addition, this mutation was changed the amino acid from methionine (AUG) to valine (GUG). Previous studies reported that Ongole grade cattle (*Bos indicus*) and Bali cattle (*Bos javanicus*) was monomorphic with CC genotype as the common genotype (Table 2). Despite, the C allele was the dominant allele in some Bos indicus cattle such as Pesisir, Najdi and Kedah-Kelantan cattle. It was concluded that monomorphism in the IGF1/SnaB1 gene in this study can be caused by the evidence of material genetics of *Bos javanicus* and *Bos indicus* in the Pasundan cattle. This finding is important to clarify the origin of Pasundan cattle according to the previous study. The IGF1/*Sna*BI gene did not used as molecular selection in this study because of monomorphic. Absence of T allele in Pasundan cattle can be caused by selection, migration and inbreeding (Bourdon 2000).

Table 1. The statistical analysis of IGF1/*Sna*B1 gene in Pasundan cattle

Genotype frequency (N)			Allele frequency		H_o	H_e	n_e	PIC	χ^2
TT	TC	CC	T	C					
0.00 (0)	0.00 (0)	1.00 (90)	0.00	1.00	0.00	0.00	1.00	0.00	-

N: number of observation; H_o: observed heterozygosity; H_e: expected heterozygosity; n_e: number of effective allele; PIC: polymorphic informative content; χ^2: Chi-square value

Table 2. The genotype and allele frequencies of IGF1/*Sna*B1 gene in the several breeds cattle

Breeds	Species	N	Genotype frequency			Allele frequency		Reference
			TT	TC	CC	T	C	
Angus	*Bos taurus*	760	0.43	0.41	0.16	0.64	0.36	Ge et al. (2001)
		204	0.19	0.52	0.29	0.45	0.55	Islam et al. (2009)
Charolais	*Bos taurus*	186	0.30	0.52	0.18	0.56	0.44	Islam et al. (2009)
Angus × Charolais	*Bos taurus*	455	0.36	0.51	0.13	0.62	0.38	Islam et al. (2009)
Hanwoo	*Bos taurus*	280	0.59	0.26	0.15	0.72	0.28	Chung & Kim (2005)
Montbeliarde	*Bos taurus*	316	0.44	0.47	0.09	0.67	0.33	Szewczuk (2016)
Friesian Holstein	*Bos taurus*	662	0.29	0.47	0.24	0.52	0.48	Siadkowska et al. (2006)
		282	0.16	0.56	0.28	0.44	0.56	Mehmannavaz et al. (2010)
		201	0.26	0.56	0.18	0.54	0.46	Szewczuk et al. (2012)
		191	0.27	0.55	0.18	0.54	0.46	Szewczuk et al. (2013)
		37	0.19	0.59	0.22	0.49	0.51	Mirzaei et al. (2012)
		848	0.33	0.48	0.19	0.57	0.43	Mullen et al. (2011)
		70	0.31	0.54	0.14	0.59	0.41	Nicolini et al. (2013)
		50	0.12	0.80	0.08	0.48	0.52	Ardicli et al. (2018)
South Anatolian Red	*Bos taurus*	50	0.08	0.30	0.62	0.23	0.77	Akis et al. (2010)
East Anatolian Red	*Bos taurus*	50	0.18	0.40	0.42	0.38	0.62	Akis et al. (2010)
Beefmaster	*Bos taurus*	25	0.00	0.07	0.93	0.03	0.97	Reyna et al. (2010)
Najdi	*Bos indicus*	84	0.02	0.83	0.15	0.10	0.90	Yazdanpanah et al. (2013)
Ongole grade	*Bos indicus*	55	0.00	0.00	1.00	0.00	1.00	Anggraeni et al. (2017)
Pesisir	*Bos indicus*	183	0.01	0.01	0.98	0.02	0.98	Yurnalis et al. (2017)
Kedah-Kelantan	*Bos indicus*	46	0.07	0.13	0.80	0.13	0.87	Suriaty et al. (2010)
Bali	*Bos javanicus*	242	0.00	0.00	1.00	0.00	1.00	Mu'in (2010)
Brahman × Charolais	*Bos ind* × *Bos tau*	55	0.02	0.44	0.54	0.24	0.76	Jeanmas et al. (2013)

N: Number of observation

```
   Forward >>>
 1 attacaaagc tgcctgcccc ccaggttcta ggaaatgaga tcatttccct cacttggcac
61 caggacgagg ggtcatccca gcgctgtctt ccattctagt ttaccccagt cgtttgaggg
121 ttaaaatcat agagtaggct tgagatggtc ttttttttcat ttcttgtttt ttaaattttg
181 tgttggctct ggaatataaa attgctcgcc catcctcyac*gtatattcct ttcatacggg
241 taaggt
   <<< Reverse
```

Figure 1. The target sequence of IGF1 gene in the exon 1 along 246 bp (GenBank: KF202095) and primer position (underlines). *) *Sna*BI restriction point; Y= T/C.

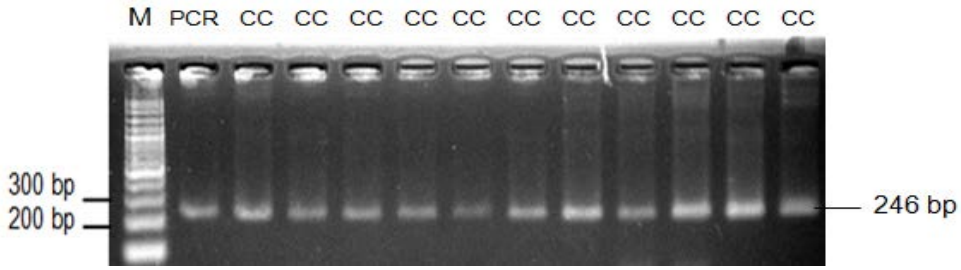


Figure 2. Visualization of IGF1/*Sna*B1 genotype in Pasundan cattle on 2% agarose gel was monomorphic with CC genotype (246 bp) as the common allele. M: DNA ladder 100 bp; PCR: DNA amplification / PCR product (246 bp).

According to Table 2, most of the IGF1/*Sna*BI gene in *Bos taurus* cattle was polymorphic and can be used for molecular selection. Chung & Kim (2005) reported that the CC genotype in Hanwoo cattle had lowest of weaning weight rather than other genotypes. Mirzaei et al. (2012) reported that the CC genotype in FH cattle had highest of serum concentration of IGF1 at 14 days prepartum in rather than other genotypes. Despite, CC genotype in FH cattle had lowest of service per conception (S/C) and days open (DO) values (Ararouti et al. 2013). Li et al. (2004) reported that CC genotype in Angus cattle had highest of body weight and weight gain rather than other genotypes. In addition, CC genotype in Polish Holstein had higest of body weight at two months and average daily gain from one to two months (Szewczuk et al. 2013). Ardicli et al (2018) reported that the CC genotype had the best of meat colour score at 24 hours post-slaughtering in FH bull. The CC genotype in the present study was detected in all samples and can be suggested as the common genotype for Pasundan cattle.

The further study regarding to detect SNP along IGF1 gene is important for developing marker assisted selection (MAS) to improve productivity traits of Pasundan cattle in the future. Several studies reported that the polymorphism of bovine IGF1 gene were occured in exon 4 and had associated with production traits in Bali cattle (Maskur et al. 2012). Mullen et al. (2011) reported that polymorphisms of bovine IGF1 gene were occured in 5' UTR, intron and 3' UTR. Polymorphism in the intron 4 of IGF1 gene was not associated with production traits in mixed population of Charolais and Beefmaster cattle (Reyna et al. 2010). Morever, polymorphism in the 5'UTR of IGF1 gene was associated with milk yield and milk quality in Polish Friesian Holstein cows (Szewczuk et al. 2013). Despite, Szewczuk (2016) reported that exon 7, exon 12 and exon 21 of bovine insulin-like growth hormon factor 1 receptor (IGF1R) was polymorphic and potential as molecular selection.

CONCLUSION

The IGF1/*Sna*BI gene of Pasundan cattle was monomorphic with C allele as the common allele in the animal studied. Therefore, this gene can not used as molecular selection for productivity traits of Pasundan cattle.

REFERENCES

Akis I, Oztabak K, Gonulalp I, Mengi A, Un C. 2010. IGF-1 and IGF-1R gene polymorphisms in East Anatolian Red and South Anatolian Red Breeds. Russ J Genet. 46:439-442.

Anggraeni A, Talib C, Asmarasari SA, Herawati T, Andreas E. 2017. Genetic polymorphisms of IGF1, GH, and OPN genes in crosses Peranakan Ongole cattle based on birth type in Central Java. JITV. 22:165-172.

Ararouti T, Abdolah M, Hasan S. 2013. Assessment of single nucleotide polymorphism in the 5'-flanking

region of insulin-like growth factor-1 (IGF-1) gene as potential marker for fertility in Holstein dairy cows. [accessed September 12th 2018]. http://www.vetjournal.istanbul.edu.tr/fulltexthtml/2013-2/5.htm.

Ardicli S, Samli H, Dincel D, Ekiz B, Yalcintan H, Vatansever B, Balci F. 2018. Relationship of the bovine IGF1, TG, DGAT and MYF5 genes to meat colour, tenderness and cooking loss. J Hellenic Vet Med Soc. 69:1077-1087.

Bourdon RM. 2000. Understanding animal breeding. New Jersey (UK): Prentice-Hall Inc.

Chung ER, Kim WT. 2005. Association of SNP marker in IGF-I and MYF5 candidate genes with growth traits in Korean cattle. Asian-Aust J Anim Sci. 18:1061-1065.

Etherton TD. 2004. Somatotropic function: The somatomedin hypothesis revisited. J Anim Sci. 82:239-244.

Ge W, Davis ME, Hines HC, Irvin KM, Simmen RCM. 2001. Association of a genetic marker with blood serum insulin-like growth factor-1 concentration and growth traits in Angus cattle. J Anim Sci. 79:1757-1762.

Indrijani, Johar A, Dudi, Wendry SP, Romi Z, Hilmia. 2012. Study identification of native cattle at West Java for meat self-sufficiency. Research Report. West Java (Indones): Department of Livestock and Food Self-Sufficiency of West Java Province.

Islam KK, Vinsky M, Crews RE, Okine E, Moore SS, Jr Crews DH, Li C. 2009. Association analyses of a SNP in the promoter of IGF1 with fat deposition and carcass merit traits in hybrid, Angus and Charolais beef cattle. Anim Genet. 40:766-769.

Jeanmas A, Sornthep T, Wiriya L, Panwadee S. 2013. Association between IGF1 gene polymorphisms and carcass traits among Thai Native, Brahman and Charolais. Agric Sci J. 44:171-174.

Li C, Basarab J, Snelling WM, Benkel B, Murdoch B, Hansen C, Moore SS. 2004. Assessment of potential candidate gene myf5 and IGF1 for growth on bovine chromosome 5 in commercial line of *Bos taurus.* Anim Sci. 82:1-7.

Maskur, Arman C, Cece S, Gunardi E, Muladno. 2012. A novel single nucleotide polymorphism in exon 4 of insulin-like growth factor-1 associated with production traits in Bali cattle. Med Pet. 35:96-101.

Mehmannavaz Y, Cyrus A, Mortaza B, Rasoul VT. 2010. Association of IGF-1 gene polymorphism with milk production traits and paternal genetic trends in Iranian Holstein bulls. Afr J Microbiol Res. 4:110-114.

Miller JR, Thomsen PD, Dixon SC, Tucker EM, Konfortov BA, Harbitz I. 1991. Synteny mapping of the bovine IGHG2, CRC and IGF-1 genes. Anim Genet. 23:51-58.

Mirzaei M, Sharifiyazdi H, Ahmadi MR, Ararooti T, Ghasrodashti AR, Kadivar A. 2012.The effect of polymorphism in gene of insulin-like growth factor - I on the serum periparturient concentration in Holstein dairy cows. Asian Pac Trop Biomed. 2:763-769.

Mu'in AM. 2010. PCR-RFLP analysis of insulin-like growth factor - I in Bali cattle. JIP. 5:1-4.

Mullen MP, Lynch CO, Waters SM, Howard DJ, Boyle PO, Kenny DA, Buckley F, Horan B, Diskin MG. 2011. Single nucleotide polymorphism in the growth hormone and insulin-like growth factor-1 genes are associated with milk production, body condition score, and fertility traits in dairy cows. Genet Mol Res. 10:1819-1830.

Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. New York (USA): Oxford University Press.

Nicolini P, Mariana C, Ana M. 2013. A polymorphism in the insulin-like growth factor 1 gene is associated with post partum resumption of ovarian cyclicity in Holstein-Friesian cows under grazing conditions. Act Vet Scand. 55:1-8.

Reyna XFDR, Montoya HM, Castrellon VV, Rincon AMS, Bracamonte MP, Vera WA. 2010. Polymorphisms in the IGF1 gene and their effect on growth traits in Mexican beef cattle. Genet Mol Res. 9:875-883.

Rose MT. 2002. The somatotropic axis of the dairy cows revisited. Anim Sci J. 73:13-19.

Siadkowska E, Lech Z, Jolanta O, Nina S, Emilia B, Jozef K. 2006. Effect of polymorphism in IGF-1 gene on production traits in Polish Holstein-Friesian cattle. Anim Sci Pap Rep. 24:225-237.

Suriaty R, Mastura Y, Mohd HAR, Mohd HA. 2010. Polymorphism of insulin-like growth factor 1 gene in Kedah-Kelantan cattle using PCR-RFLP technique. Malaysian J Vet Res. 6:31-36.

Szewczuk M, Slamowir Z, Ewa CP, Jerzy W. 2012. Association between IGF1R/i16/TaqI and IGF1/SnaBI polymorphism and milk production traits in Polish Holstein-Friesian cows. Anim Sci Pap Rep. 30:13-24.

Szewczuk M, Bajurna M, Zych S, Kruszynski W. 2013. Association of insulin-like growth factor I gene polymorphisms (IGF1/TasI and IGF1/SnaBI) with the growth and subsequent milk yield of Polish Holstein-Friesian heifers. Czech J Anim Sci. 58:404-411.

Szewczuk M. 2016. Association of single nucleotide polymorphisms in genes coding insulin-like growth factor 1 system and milk production traits in Montbeliarde cows. S Afr J Anim Sci. 46:191-195.

Yurnalis, Arnim, Dino EP. 2017. Polymorphism of insulin-like growth factor 1 gene (IGF1/TasI, IGF1/SnaBI, IGF1/RsaI) and the association with daily gain of Pesisir cattle local breed from West Sumatera, Indonesia. Pak J Biol Sci. 20:210-216.

Yazdanpanah A, Hedayatollah R, Khalil M, Morteza M, Saber K. 2013. Polymorphism of insulin-like growth factor 1 in Najdi cattle populations. American J Biochem Biotech. 9:300-306.

Serum Biochemical, Hormonal and Fatty Acid Profiles During the Late Gestation of Pregnancy Ketosis in Boer Cross Goats

Affan AA[2], Amirul FMA[2], Ghani AAA[2], Annas S[1,3], Zamri-Saad M[1,3], Hassim HA[1,2]

[1]*Research Centre for Ruminant Disease*
[2]*Department of Veterinary Preclinical Sciences*
[3]*Department of Veterinary Laboratory Diagnostic, Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor*
E-mail: haslizaabu@upm.edu.my

(received 14-11-2018; revised 26-11-2018; accepted 11-12-2018)

ABSTRAK

Affan AA, Amirul FMA, Ghani AAA, Annas S, Zamri-Saad M, Hassim HA. 2018. Profil biokimiawi serum, hormon dan asam lemak selama fase akhir kebuntingan ketosis pada kambing Boer persilangan. JITV 23(4): 180-188. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1922

Ketosis pada kehamilan merupakan salah satu penyakit metabolisme umum yang mempengaruhi produksi daging dan susu kambing. Penelitian ini menggunakan 16 ekor kambing betina bunting umur 80 hari. Sebanyak total 8 kambing betina dikategorikan sebagai kelompok kontrol (kambing betina bunting sehat) diberikan pakan rumput *Napier* dan konsentrat untuk kambing serta air secara *ad libitum*. Delapan kambing betina bunting lainnya dikategorikan ke dalam kelompok yang diberikan perlakuan yang menderita ketosis berdasarkan tanda-tanda klinis dan keberadaan badan keton pada urin. Sampel darah diperoleh dari semua kambing untuk keperluan analisis profil biokimia yaitu: glukosa, *Beta-hydroxybutyrate* (BHBA), asam lemak bebas (FFA), kalsium, elektrolit (sodium, potasium, klorida), enzim hati dan tingkat hormon (kortisol dan insulin). Setelah dilakukan penyembelihan 3 ekor kambing dari masing-masing kelompok, hatinya dikumpulkan dan kemudian dipelajari profil asam lemaknya. Hasil penelitian menunjukkan BHBA, FFA, kalsium, *transaminase aspartate* (AST), *transmirase glutamil gama* (GGT) dan hormon kortisol secara signifikan lebih tinggi pada kambing yang menderita ketosis dibandingkan dengan kontrol. Sementara itu, konsentrasi glukosa, sodium, potassium, klorida dan hormon insulin lebih rendah pada kambing yang menderita ketosis dibandingkan dengan kontrol. Selanjutnya, komposisi asam lemak dalam plasma darah kambing betina bunting dengan riwayat ketosis menunjukkan level yang lebih tinggi pada palmitat, asam stearik dan oleik, sementara itu pada hati menunjukkan nilai yang lebih tinggi untuk palmitat, asam *oleic* dan linoleik.

Kata Kunci: *Beta-Hydroxybutyrate*, Sampel Darah, Asam Lemak, Glukosa, Hati

ABSTRACT

Affan AA, Amirul FMA, Ghani AAA, Annas S, Zamri-Saad M, Hassim HA. 2018. Serum biochemical, hormonal and fatty acid profiles during the late gestation of pregnancy ketosis in Boer cross goats. JITV 23(4): 180-188. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1922

Pregnancy ketosis has been recognized as one of the common metabolic disease affecting goat's meat and milk production. For the present study, sixteen (n=16) individuals of pregnant does at day 80 of pregnancy had been used. A total of 8 does were categorized as control group (healthy pregnant goats), were fed on Napier grass and goat concentrate with water ad libitum, and another 8 does were considered as treatment group which categorized as ketosis based on the clinical signs and presence of ketone body in urine. Blood sample were collected from all goats for biochemical profiles analysis which were glucose, Beta-hydroxybutyrate (BHBA), free fatty acid (FFA), calcium, electrolytes (sodium, potassium, chloride), liver enzyme and hormonal levels (cortisol and insulin). Three does from each group were slaughtered and liver samples were collected for fatty acid profiles study. In this study, the BHBA, FFA, calcium, amino aspartate transferase (AST), gamma glutamyl transferase (GGT) and cortisol hormone were significantly higher in pregnancy ketosis goats as compared to control group. Meanwhile, the concentration of glucose, sodium, potassium, chloride and insulin hormones were lower in pregnancy ketosis goats as compared to control. Furthermore, the fatty acid composition in blood plasma of pregnant goat with ketosis showed higher level of palmitic, stearic and oleic acid, while in liver, palmitic, oleic and linoleic acid was found higher.

Key Words: Beta-Hydroxybutyrate, Blood Sample, Fatty Acid, Glucose, Liver

INTRODUCTION

There has been a sharp increase in the demand for goat's milk and meat in Malaysia, particularly in the last three decades due to rapid economic and population growth, with the resultant effects of urbanization, income growth and changing consumer preference (Bisant 2010). Nevertheless, scientifically based information on goat farm and industry in Malaysia is extremely limited to complement the sudden surge of

demand for goat's milk and meat. Among the urgent issues faced by goat farmers include 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 disease affecting goat's meat and milk production (Bani Ismail et al. 2008). The condition has been observed in Malaysia but not being reported. In Faculty of Veterinary Medicine, UPM, most of the farmer of goat's farm under the "Program Ladang Angkat Fakulti' has reported that pregnancy ketosis is the common metabolic disease in the farm with the morbidity and mortality rate of 5-10% and 80%, respectively (Syahirah et al. 2015). Indeed, this has resulted in poor performance of the goat which further caused high morbidity and mortality.

Pregnancy ketosis commonly occurs in does during the late stage of gestation. The main cause of pregnancy ketosis in goat is a disturbance of carbohydrate metabolism due to the high demands for glucose by the developing fetuses in the last trimester of pregnancy, resulting in negative energy balance (Schlumbohm & Harmeyer 2004). Clinical signs, often with a slow onset, are characterized by separating of the flock, anorexia, locomotion disorders and neurological signs like bilateral blindness, teeth grinding and muscle tremors. While morbidity is low, the reported mortality in absence of therapy for pregnancy ketosis is 90% (Schlumbohm & Harmeyer 2004). It is biochemically characterized by hypoinsulinemia, hypokalemia and high levels of ketone bodies found in the plasma of does diagnosed with pregnancy ketosis (Van Saun 2000). The serum glucose level decreases which causes the breakdown of triglycerides into fatty acids and glycerine mobilizes from the fat reserves. Due to the excessive degradation of fat reserves, β-hydroxybutyrate acid (BHBA), acetoacetate and acetone are produced. A variety of metabolic disorders may occur during ketosis from the examination of energy metabolism, oxidative stress clinical pathology and immune function.

There are a lot of studies related to pregnancy ketosis in goats. However, there are still lacks of information available on the diagnosis of clinical and serobiochemical study as well as hormonal status conducted on goats in Malaysia. Therefore, this study was carried out to assess the effect of pregnancy ketosis in Boer cross goats during late trimester on clinical and serobiochemical parameters, endocrine response (changes in the insulin and cortisol hormones) as well as changes in electrolytes concentration.

Thus, the objectives of this research were to determine the serum biochemical and hormonal profiles in does with pregnancy ketosis, and to assess the fatty acids composition in the blood and liver of does with pregnancy ketosis.

MATERIALS AND METHODS

Animal induction and selection of pregnancy ketosis

Sixteen pregnant goats that are in the last trimester were used in this study. All goats were divided randomly into control (n=8) and treatment (n=8) groups. The animals in control and treatment group were placed into different pen. The pregnant goats in control group were fed based on the standard feeding requirement which enough to meet nutrient requirement energy for pregnant goats (602.5 kJ ME/body weight kg/day) (NRC 2007). The amount of feed given to control group were 70% of Napier grass (dry matter: 10.57%, crude protein: 14.62%, crude fibre: 30.39%, energy: 8.30 MJ/kg) and 30% of goat concentrate (moisture: 13%, crude protein: 14.5%, crude fiber: 20%, dry matter: 87%, ash: 10%, crude fat: 6.65%, energy: 10.41%). Water was given *ad libitum* during experiment. For treatment group, they are induced with ketosis by restricted the energy intake for up to 50% of the daily requirement.

All procedures and techniques related to these does for research, and the experimental design were undertaken following the guidelines of the Research Policy of the Universiti Putra Malaysia. The animal utilization protocol and sampling procedure was approved by Ethical Committee for Animal Experiments, Universiti Putra Malaysia (UPM/IACUC/AUP-R071/2016).

Blood collection

All does were bled in the morning prior to feeding at 8.00 am and the blood sample were transported to the Physiology Laboratory by using ice boxes to avoid blood haemolysed. Blood sample were collected from jugular vein of both control and treatment goats with a proper restrain after clinical examination. The blood was collected by using needle size 21G and the sample was collected in Vacutainer and EDTA tubes. Plasma were harvested from EDTA tube by separating the blood using centrifuge (SH120-II) under 3000x rpm for 15 minutes. All plasma was stored at -80°C until further used. Furthermore, 1 ml of plasma was collected and kept in -20°C for fatty acid analysis.

Serobiochemical assay

Blood samples were used for serobiochemical analysis which was glucose, calcium, BHBA, sodium, chloride and potassium by using chemistry analyzer (Siemens Dimension Xpand Plus, USA). Commercial ELISA kits were used to determine BHBA and free fatty acids. All parameters were determined from

diluted plasma samples with buffer recommended in the commercially available ELISA kits.

Hormonal assays

Cortisol and insulin levels were determined by using plasma sample which represent in duplicate. Cortisol antiserum was obtained from Cortisol EIA Kit. The inter and intra assay coefficients was 6.7% and 1.1% respectively. All hormones levels were determined from diluted plasma samples with buffer recommended in the commercially available ELISA kits.

Liver collection

Three goats from each treatment and control group were slaughtered according to the Islamic traditions (Halal Slaughter Method) by severing the jugular veins, carotid arteries, trachea and esophagus (Ahmed et al. 2015). The liver organ was collected and cut into dice at randomly part on the liver after the slaughtering process. The samples were wrapped in aluminum foil, placed in polyvinyl chloride (PVC) plastic bags and stored at -80°C until further analysis for total FFA extraction.

Fatty acid profile determination

Chemical and glassware

All apparatus such as methylation tubes, screw caps and extraction tube stoppers were soaked for two hours in Decon 90 (Decon Laboratories Ltd., Sussex UK), before and after the fatty acid extractions. Then, all apparatus was soaked in distilled water overnight and rinsed again before oven dried at 60°C. Other glassware such as separating flasks, funnels, extraction tubes and round bottom flasks were washed in an automated laboratory glassware washer using acid and alkaline washes for about three hours. All chemicals, solvents and laboratory supplies used for total lipid extraction and preparation of fatty acid methyl esters (FAME) were of analytical grade. All chemicals and solvents were free from contamination with rubber or fat derivatives.

Total lipid extraction

Based on the method by Folch et al. (1957), the total fatty acids were extracted from plasma and liver tissues using chloroform: methanol 2 : 1. The blood plasma samples were extracted differently, where 1.0 to 2.0 ml of the sample was aspirated into 50 ml stoppered ground-glass extraction flask containing 40 ml of chloroform-methanol (2 : 1, v/v). The tube was gases with nitrogen, stoppered and then vigorously shakes before allowed to stand for 12 hours. The mixture containing the extracted fatty acids was filtered through a No. 1 Whatman paper (Whatman International Ltd., Maidstone, England) into a 250 ml separating flask using a filter funnel. The paper was washed with 10 ml of fresh chloroform-methanol (2 : 1, v/v). Ten ml of normal saline solution were added to facilitate phase separation. The mixture was then shaken vigorously for one minute and was left to stand for four hours. After this washing phase, the lower phase contained 86 parts chloroform: 14 parts methanol: 1part water (Shahidi & Wanasundara 1998). The upper phase would contain 3:48:47 parts of chloroform, methanol and water respectively. After complete separation at the end of fourth hour, the upper phase was discarded and lower phase was collected in a round bottom flask and evaporated by rotary evaporation (Heidolph GmBH, Germany) at 700°C. The total lipid extract was then immediately transferred to a capped methylation tube by re-diluting it with five ml fresh chloroform-methanol (2 : 1, v/v).

Furthermore, for liver sample, about 1 g of tissues were cut and homogenized in 40 ml of chloroform:methanol (2:1, v/v) using an Ultra-Turrax T5 FU homogenizer (IKA Analysentechnik GmBH, Germany) without thawing (Shahidi & Wanasundara 1998) within a 50 ml stoppered ground-glass extraction tube.

Preparation of fatty acid methyl esters

Transmethylation of the extracted fatty acids to fatty acid methyl esters (FAME) were carried out using 14% methanolic boron trifluoride (BF3) according to methods in AOAC (2007). The internal standard, heneicosanoic acid (21 : 0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample prior to transmethylation to determine the individual fatty acid concentrations within the samples.

The sample extract was then dried on a heating block (40°C) under a constant and mild flow of pure nitrogen gas. After drying the chloroform: methanol, two ml of 0.66 N methanolic potassium hydroxide (R & M Chemicals, Essex, U.K.) was added to saponify the lipid sample. The methylation tube was flushed with nitrogen, stoppered and heated in a boiling water bath for 10 min with occasional shaking. After the mixture had cooled down, two ml of 14 % boron trifluoride (BF3) (Sigma Chemical Co., St. Louis, Missouri, USA) were added to initiate trans-esterification and the mixture was reheated for 20 min in a boiling water bath (Rajion 1985).

After cooling, four ml of distilled water and four ml of petroleum ether (boiling point 40-60°C) were added and the mixture was vortexed for 60 sec. The mixture was then centrifuged at 1500G for 10 minutes to

increase phase separation. The upper petroleum phase was transferred to another test tube using pasture capillary pipettes and washed with one ml of distilled water to remove residual BF3. The upper phase from this test tube was then transferred accurately again to a second test tube and 0.5 g anhydrous sodium sulphate (R & M Chemicals, Essex, U.K.) was added to dry the sample and remove any residual water. Finally, the petroleum ether containing the FAME was transferred to a four ml screw-capped vial (Kimble Glass Inc., USA), flushed with nitrogen, closed tightly and stored at 4°C until analysis by gas-liquid chromatography.

Gas liquid chromatography

The methyl esters were quantified by GC using a 30m x 0.25mm ID (0.20 μm film thickness). One microlitre was injected by an auto sampler into the chromatograph, equipped with a split/splitless injector and a FID detector. High purity hydrogen (Dominick Hunter, Parker Hannifin ltd, UK) and compressed air (Malaysian Oxygen Bhd., Malaysia) were used for the flame ionization detector in the gas-liquid chromatograph. The injector temperature was programmed at 250°C and the detector temperature was 300°C. The column temperature program initiated runs at 100°C, for 2 min, warmed to 170°C at 10°C /min, held for 2 min, warmed to 220°C at 7.5°C /min, and then held for 20 min to facilitate optimal separation.

Identification of fatty acids was carried out by comparing relative FAME peak retention times of samples to standards obtained from Sigma (St. Louis, MO, USA). Both gravimetric calculations and normalised percentage (%) of total Fatty acid (FA) were used to determine the differences in FA composition. Peak areas were determined and calibrated using a personal computer integrator (Hewlett-Packard, Avondale, PA). Automatic expression of the peak areas as absolute and percentage amount of a detected fatty acid was obtained with a programmed PC under Microsoft Excel 2000 (Microsoft Corp., Redmond, USA).

The amount of fatty acid is determined by their relative proportions (normalized percentages to total fatty acids) (Huerta-Leidenz et al. 1991; Alfaia et al. 2006). The normalised percentages describe the interactive and comparable relationship among fatty acids regarding lipid quality, while the gravimetric concentration can show the actual amount of fatty acids in tissues, which relates to nutritional intake.

Data analysis

All the analysis was done with SPSS version 22.0 software. The data was analyzed using independent T-test to compare between means of different groups. Data represent as a mean were considered significantly different when $P<0.05$.

RESULTS AND DISCUSSION

All goats in treatment group showed more than three clinical signs of pregnancy ketosis which appear at 3 to 6 days of induction with pregnancy ketosis in the form of anorexia, dullness, teeth grinding and weakness. However, for control group which were healthy pregnant goats, there were no clinical signs shown throughout the experiment.

Pregnancy ketoses in goats are due to the animals failed to meet the energy demand for fetal unit. The diagnosis of pregnancy ketosis is based on the physical signs, hematological and biochemical measures. Based on the observation in treatment group, depression and teeth grinding were the first clinical signs. A study by Barakat et al. (2007) reported that anorexia and sternal recumbence were observed during ketosis state of pregnancy goat. Indeed, the clinical signs that showed by the pregnancy ketosis goats in this study are similar with the previous study. These clinical signs are caused by negative energy balance due to the lack of energy which is low energy intake. As a result, it will lead the animals become recumbence and lack of energy to support daily activities.

In regard to biochemical analysis, the value of glucose, BHBA, FFA, calcium, AST, GGT and cortisol hormone were significantly higher in treatment group ($P<0.05$) than those of control as shown in Table 1 and Figure 1. However, insulin hormone was shown significantly lower in concentration in treatment group ($P<0.05$) when compared to control (Figure 2). The values of sodium, potassium, and chloride were not significantly different between treatment groups than those of control (Table 1).

For serobiochemical profiles, it was characterized as subclinical pregnancy ketosis based on the FFA result. According to Brozos et al. (2011). The level of FFA in the blood can be categorized in three part which are; (healthy pregnant goats = 0.00 – 0.79 mmol/L; subclinical pregnancy ketosis = 0.80 – 0.99 mmol/L; clinical pregnancy ketosis = 1.00 – 3.00 mmol/L). In present study, there were elevation of FFA and BHBA during decrement of glucose value in ketosis goats. It is similar with the study by Schlumbohm & Harmeyer (2003), which stated that increment of BHBA values resulted in a significant drop of glucose turnover. According to Barakat et al. (2007), the increasing of FFA concentration in plasma of affected goats could be attributed to the increasing mobilization of fatty acids from the adipose tissue in response to a requirement for endogenous substrate for energy production during pregnancy. In other words, the elevation level of FFA

due to ketosis inside plasma reflected the formation of fatty liver in pregnant does.

Furthermore, elevation of BHBA level also able to inhibit the hepatic gluconeogenesis, which could increase the maternal hypoglycaemia (Schlumbohm & Harmeyer 2004). Besides, the study showed that the glucose level for ketotic goats was lower than healthy goats. This is because glucose is the main source of energy supply in the body. Indeed, glucose level shows relation to the animal status which values related falling with a negative energy balance.

In this study, there was declining of calcium level in the blood (hypocalcemia) with slightly locomotion disturbance in the pregnancy ketosis goats. A study by Henze et al. (1998) showed that elevation of ketone bodies and free fatty acid could lead to significant decreased in plasma calcium concentration. This is because elevation concentration of calcium in the circulation of pregnancy does is required for skeletal development in growing fetus especially during the last trimester (Schlumbohm & Harmeyer 2003).

Table 1. Serobiochemical profiles in control vs treatment groups

Parameters	Control Group Healthy Pregnant Goat	Treatment Group Pregnancy Ketosis Goat	Standard Normal Range
Glucose (mmol/L)	2.88±0.11[a]	1.13±0.33[b]	2.70-4.20
Beta- Hydroxybutyrate (mmol/L)	0.13±0.01[a]	1.37±0.11[b]	0.10-0.70
Free Fatty Acid (mmol/L)	0.18±0.02[a]	0.98±0.05[b]	0.00-0.79
Calcium (mmol/L)	2.24±0.07[a]	1.83±0.06[b]	2.20-3.20
Sodium (mmol/L)	143.60±0.82[a]	143.80±1.15[a]	142.00-155.00
Chloride (mmol/L)	104.80±0.74[a]	103.60±2.31[a]	99.00-100.00
Potassium (mmol/L)	4.54±0.09[a]	4.18±0.05[a]	3.50-6.70
Amino aspartate transferase (U/L)	87.29±2.46[a]	104.00±0.09[b]	50.00-100.00
Gamma glutamyl transferase (U/L)	39.00±1.67[a]	48.06±1.87[b]	30.00-50.00

Note: (Means±SEM) in goats with pregnancy ketosis (n=8) and control goat (n=8); [a,b]different letters between columns denote significance (P<0.05)

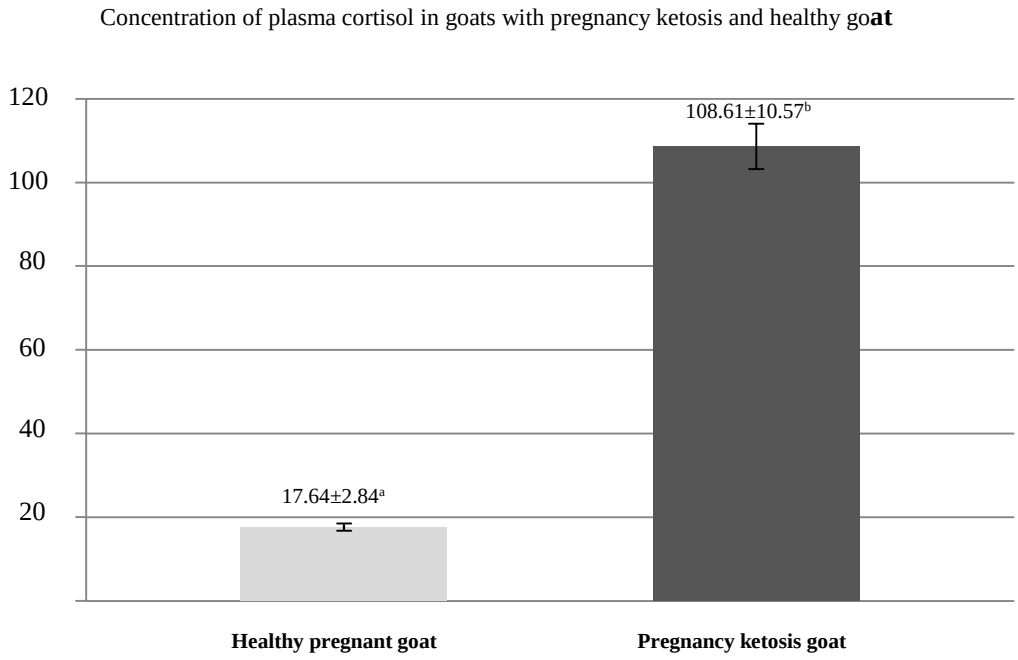


Figure 1. Concentration of plasma cortisol hormone in goats with pregnancy ketosis and healthy goat.

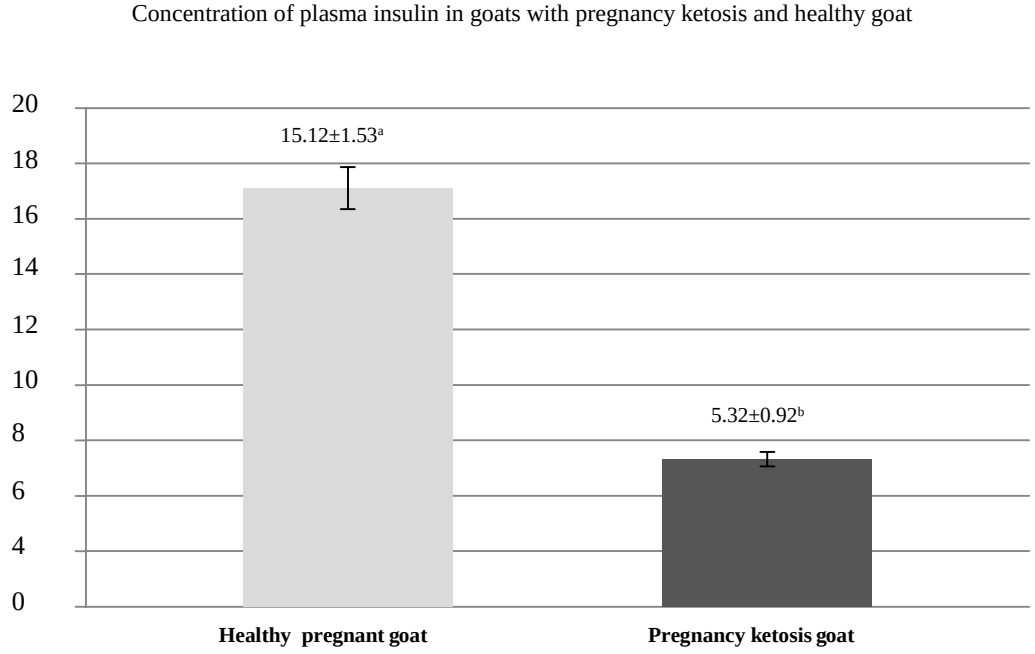


Figure 2. Concentration of plasma insulin hormone in goats with pregnancy ketosis and healthy goat.

In treatment group, there were elevation of AST and GGT concentration recorded, which indicated that there was liver damage in ruminant (Barakat et al. 2007). According to study by Abd-Elghany et al. (2007), it was reported that significant elevation of AST and GGT levels could throw some diagnosis on the hepatic influence of caprine pregnancy ketosis which attributed to fat mobilization and associated with an increasing of FFA in plasma.

According to Halford & Sanson (1983), electrolytes in the blood of pregnancy ketosis in ewes were decreased in concentration; however, no differences of electrolyte concentration were observed between treatment and control group in this study. It is indicated that there could be a tremor in the electrolytes and some minerals which may be essence to dehydration, stress of starvation, and kidney involvement in the pathogenesis of goat pregnancy ketosis (Judith & Thomas 1988). Based on the result, there is high level of cortisol in treatment group compared to control group. These findings are supported by Moyes et al. (2009) which reported that higher serum cortisol concentration present in negative energy balance ruminant animal as compared to animal with an *ad libitum* diet. As mentioned by Kristina et al. (2010), cortisol is one of the hormones used as an indicator of stress and pain during ketosis state in ruminant.

Apart from that, insulin is one of important hormones in regulation of energy homeostasis which can alter fatty acid release and ketogenic process (Abd-Elghany et al. 2010). Insulin secretion plays roles in regulating the utilization of ketone bodies, and uptake of BHBA as well as acetate. In this study, the lower insulin value was observed in treatment group (5.32±0.92 μU/ml) as compared to the control (15.12±1.53 μU/ml). The result indicated that significant decrement of insulin values in treatment group may refer to the fact that insulin may have potential in inhibitory role of ketogenesis (Abd-Elghany et al. 2010). As mentioned by Ganong (2007), rising rate of lipolysis and production of ketone bodies are caused by deficiency of insulin. This theory is in line with the finding of this study which FFA and BHBA concentration was high while the insulin level was low during ketosis state. Apart from that, insulin is one of important hormones in regulation of energy homeostasis which can alter fatty acid release and ketogenic process (Abd-Elghany et al. 2010). Insulin secretion plays roles in regulating the utilization of ketone bodies, and uptake of BHBA as well as acetate.

Fatty acid composition in blood

The composition of fatty acids in blood was tabulated in Table 2. In general, most of the fatty acids composition in treatment group showed a significant higher concentration as compared to control group except for alpha linoleic acid (C18:3) and docosapentanoic acid (C22:5) (Table 2). Among these fatty acids, the palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and docosahexanoic acid (C22:6) in treatment group showed 3 to 4 fold high concentration as compared to the control group (palmitic acid: 14.62±0.98mg/dL vs 5.33±0.87 mg/dL, stearic acid: 16.18±1.17mg/dL vs 5.83±1.12 mg/dL, oleic acid: 27.95±1.88 mg/dL vs 6.90±1.18 mg/dL, linoleic acid: 13.58±1.19 mg/dL vs 6.26±1.47 mg/dL and docosahexanoic acid: 13.97±1.86 vs 5.99±1.23 mg/dL) (Table 2).

Fatty acid composition in liver

Most of the fatty acids composition in the liver of treatment group showed a significant high concentration as compared to the control group, except for eicosapentanoic acid (C20:5) (Table 3). The higher composition of fatty acids in the liver for both groups was palmitic acid (C16:0), linoleic acid (C18:2) and oleic acid (C18:1) while the lower composition of fatty acids were Alpha Linoleic acid (C18:3) and Docosapentanoic acid (C22:5).

In the current study, results showed that fatty acids compositions in treatment group were high as compared to the control. This could be due to different adaption of physiological state and body needs particularly of energy demand in both groups. Indeed, high concentration of fatty acids in blood observed during ketosis condition may cause by lipolysis occurred in adipocyte cell. A study by Nogalski et al. (2012), reported that most of fatty acids present in blood during negative energy balance state caused by lipolysis originated from subcutaneous adipose tissue.

Based on the results, there are three types of fatty acids that showed higher concentration in treatment group which are palmitic acid (C`16:0), stearic acid (C18:0), and oleic acid (C18:1). These three fatty acids showed an increment up to 3 to 4 folds as compared to the control, which indicating that the lipolysis process has been occurred extensively during inadequate of energy condition in pregnancy ketosis. Based on the previous study by Mansbridge & Blake (1997), those fatty acids were also found increased in the blood stream which arise from a source other than *de novo* synthesis in the liver of ruminant.

In this current study, fatty acids composition in the liver of treatment group was also found higher as compared to control group. Indeed, some of the fatty acids such as palmitic acid (C 16:0), oleic acid (C 18:1), and linoleic acid (C 18:2) showed an increment up to 5 – 7 folds in comparison to control animals. The increment of these fatty acids concentration in liver could also be observed in the blood as discussed earlier. The sudden increase in the concentration of fatty acids content in the blood as well as in the liver in pregnancy ketosis goat could be attributed to the increased mobilization of fatty acids from adipocyte and undergo oxidization process in liver, responses to an increased requirement for endogenous substrate for energy

Table 2. Fatty acid composition in the blood of control and treatment group

Fatty acid composition		Control Group (mg/dL) (n=8)	Treatment Group (mg/dL) (n=3)	Changes
Palmitic acid	C16:0	5.33±0.87[a]	14.62±0.98[b]	↑
Palmitoleic acid	C16:1	0.45±0.08[a]	0.83±0.10[b]	↑
Stearic acid	C18:0	5.83±1.12[a]	16.18±1.17[b]	↑
Oleic acid	C18:1	6.90±1.18[a]	27.95±1.88[b]	↑
Linoleic acid	C18:2	6.26±1.47[a]	13.58±1.19[b]	↑
Alpha Linoleic acid	C18:3	0.42±0.08[a]	0.51±0.07[a]	▬
Arachidonic acid	C20:4	1.62±0.28[a]	3.49±0.29[b]	↑
Eicosapentanoic acid	C20:5	0.25±0.05[a]	0.82±0.17[b]	↑
Docosapentanoic acid	C22:5	0.41±0.06[a]	0.42±0.08[a]	▬
Docosahexanoic acid	C22:6	5.99±1.23[a]	13.97±1.86[b]	↑

Notes: All value express in (means±SE) mg/dL
Control group = healthy pregnant goat
Treatment group = pregnancy ketosis goat
a,b different letters between columns denote significance (P<0.05)
↑ = increasing of fatty acid composition in the blood
▬ = no changes of fatty acid composition in the blood

Table 3. Fatty acid composition in the liver of control and treatment group

Fatty acid composition		Control Group (mg/dL) (n=8)	Treatment Group (mg/dL) (n=3)	Changes
Palmitic Acid	C16:0	39.20±3.58[a]	207.00±8.91[b]	↑
Palmitoleic Acid	C16:1	4.49±1.30[a]	11.02±0.60[b]	↑
Stearic Acid	C18:0	50.66±2.15[a]	92.10±14.26[b]	↑
Oleic Acid	C18:1	62.65±4.57[a]	454.20±19.32[b]	↑
Linoleic Acid	C18:2	28.49±1.88[a]	130.60±6.21[b]	↑
Alpha Linoleic Acid	C18:3	0.85±0.16[a]	2.89±0.25[b]	↑
Arachidonic Acid	C20:4	26.43±1.65[a]	46.86±1.82[b]	↑
Eicosapentanoic Acid	C20:5	2.26±0.12[a]	6.63±2.05[b]	↑
Docosapentanoic Acid	C22:5	1.82±0.12[a]	3.42±0.28[b]	↑
Docosahexanoic Acid	C22:6	12.31±1.78[a]	75.67±5.06[b]	↑

Notes: All value express in (means±SE) mg/dL
Control group = healthy pregnant goat
Treatment group = pregnancy ketosis goat
a,b different letters between columns denote significance ($P<0.05$)
↑= increasing of fatty acid composition in the liver

production during last trimester of pregnancy (Noble et al. 1971). In addition, during ketosis state, high amount of fatty acid delivered into mitochondria in the liver and fatty acid oxidation-derived acetyl –CoA is diverted from Kreb cycle to ketogenesis which generating acetoacetate (AcAc) and BHBA and releasing back to blood stream (Schugar & Crawford 2012). Therefore, it concluded that lipolysis is the main cause that triggers gluconeogenesis and ketogenesis occurred during pregnancy ketosis.

CONCLUSION

In conclusion, the severity of pregnancy ketosis in goat may cause changes in serobiochemical and hormonal profiles. Pregnancy ketosis affected the liver organ when enzyme AST and GGT level were increased in the blood analysis. Moreover, muscular imbalance and weakness in pregnancy ketosis goat appeared concurrently with declining of calcium level in blood. Meanwhile, no changes in electrolytes levels and mineral balance recorded during ketosis state in pregnancy goat. Furthermore, plasma free fatty acids levels were observed to be higher in concentration of blood as well as in liver of pregnancy ketosis does. The elevation of free fatty acid was found during high level of BHBA and low glucose level in blood. Stress hormone, cortisol was found to be significantly elevated in pregnancy ketosis does which proved that pregnancy ketosis is a stressful condition that may affect the productivity of meat and milk production. Meanwhile, insulin hormone was found lower in concentration due to low of glucose levels during ketosis condition.

REFERENCES

Abd-Elghany H, Saad S, Seham Y. 2011. Hematobiochemical profile of pregnant and experimentally pregnancy toxemic goats. J Basic Appl Chem. 1:65-69.

Abd-Elghany H, Seham Y, Saad S. 2010. Some Immunohormonal changes in experimentally pregnant toxemic goats. Vet Med Int. 2010:5.

Ahmed HO, Zaiton H, Mohd NAM. 2015. Effect of slaughtering methods on meat quality indicators,chemical changes and microbiological quality of broiler chicken meat during refrigerated storage. J Agric Vet Sci. 8:12-17.

Alfaia CM, Ribeiro SS, Lourenco MRA, Quaresma MAG, Martins SIV, Portugal AP, Fontes CMGA, Bessa RJB, Castro MLF, Prates, JAM. 2006. Fatty acid composition, conjugated linoleic acid isomers and cholesterol in beef from crossbreed bullocks intensively produced and from Alentejana purebred bullocks reared according to Carnalentejana PDO specifications. Meat Sci. 72:425-436.

[AOAC] Association of Official Analytical Chemists. 2007. Official Methods of Analysis of AOAC International. 18th ed. Gaithersburg (UK): AOAC International.

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.

Barakat SEM, Al-Bhanasawi NM, Elazhari GAO, Bakhiet. 2007. Clinical and Serobiochemical Studies on Naturally Occuring Pregnancy Toxemia in Shamia Goats. J Anim Vet Adv. 6:768-772.

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

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 Pract. 27:105-113.

Folch J, Lees M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226:497-509.

Ganong WF. 2007. Review of medical physiology. 22nd ed. New York (USA): McGraw Hill.

Halford DM, Sanson DW. 1983. Serum profiles determined during ovine pregnancy toxemia. Agric Pract. 4:27-33.

Henze P, Bickhardt K, Fuhrmann H, Sallmann HP. 1998. Spontaneous pregnancy toxaemia (ketosis) in sheep and the role of insulin. J Vet Medic Series A. 45:255-266.

Huerta-Leidenz NO, Cross HR, Luntt DK, Pelton LS, Savell JW, Smith SB. 1991. Growth, carcass traits, and fatty acid profiles of adipose tissues from steers fed whole cottonseed. J Anim Sci. 69:3665-3672.

Jamaludin AA, Idris K, Roslaini R. 2012. Challenges facing dairy goat farmers in Malaysia. Abdullah R, Omar MA, Makkar H, Otte J, Rajion MA, Alimon AR, Boo LJ, Kam HA, Li CW, editors. Proceeding of 1st Asia Goat Conference. Kuala Lumpur (Malays): Universiti Putra Malaysia and The Food and Agricultural Organization of the United Nations. p. 11-13.

Judith VM, Thomas HH. 1988. Pregnancy toxemia and ketosis in ewes and does. Vet Clin North Am**:** Food Anim Pract. 4:307-315.

Kristina BF, Orjan AL, Bernt VJ. 2010. Low cortisol levels in blood from dairy cows with ketosis: a field study. Acta Veterinaria Scandinavica. 52:31.

Mansbridge RJ, Blake JS. 1997. Nutritional factors affecting the fatty acid composition of bovine milk. Br J Nutr. 78:37-47.

Moyes KM, Drackley JK, Salak-Johnson JL, Morin DE, Hope JC, Loor JJ. 2009. Dietary induced negative energy balance has minimal effects on innate immunity during a *Streptococcus uberis* mastitis challenge in dairy cows during midlactation**.** J Dairy Sci. 92:4301-4316.

[NRC] National Research Council. 2007. Nutrient Requirements of Small Ruminants: sheep, goats, cervids, and New World camelids. Washington DC (USA): National Research Council, National Academies Press.

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

Nogalski Z, Wroński M, Sobczuk-Szul M, Mochol M, Pogorzelska P. 2012. The Effect of Body Energy Reserve Mobilization on the Fatty Acid Profile of milk in High-yielding Cows. Asian-Austr J Anim Sci. 25:1712-1720.

Rajion MA. 1985. Essential fatty acid metabolism in the fetal and newborn lamb (Thesis). [Melbourne (Australia)]: The University of Melbourne. 315p.

Schlumbohm C, Harmeyer J. 2003. Hypocalcaemia reduces endogenous glucose production in hyperketonemic sheep. J Dairy Sci. 68:1953-1962.

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

Shahidi F, Wanasundara JPD. 1998. Extraction and Analysis of Lipids. In: Akoh CC, Min DB, editors. Food lipids: Chemistry, nutrition, and biotechnology. New York (USA): Marcel Dekker Inc. 914p.

Van Saun RJ. 2000. Pregnancy toxaweemia in a flock of sheep. J Am Vet Medic Assoc. 217:1536-1539.

Improvement of Viability of *Lactobacillus casei* and *Bifidobacterium longum* with Several Encapsulating Materials using Extrusion Method

Widaningrum[1], Miskiyah[1], Indrasti D[2], Hidaya HC[2]

[1]*Indonesian Centre for Agricultural Postharvest Research and Development, Bogor, Indonesia*
[2]*Department of Food Technology, Bogor Agricultural University*
E-mail: widaningrum.adnan@gmail.com

(received 23-08-2018; revised 06-12-2018; accepted 07-12-2018)

ABSTRAK

Widaningrum, Miskiyah, Indrasti D, Hidaya HC. 2018. Peningkatan viabilitas bakteri *Lactobacillus casei* dan *Bifidobacterium longum* dengan beberapa materi enkapsulasi menggunakan metode ekstruksi. JITV 23(4): 189-201. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1547

Lactobacillus casei dan *Bifidobacterium longum* merupakan bakteri probiotik yang umum digunakan sebagai *starter* kering pada sistem pangan. Proses pengeringan pada produksi *starter* kering dapat mengurangi jumlah probiotik, sehingga probiotik sangat perlu untuk dienkapsulasi. Tujuan penelitian ini yaitu untuk memperoleh bahan pengkapsul terbaik untuk melindungi kedua probiotik tersebut. Teknik enkapsulasi yang digunakan dalam penelitian ini yaitu ekstrusi pada kombinasi maltodekstrin-alginat, pati sagu-alginat, pati jagung-alginat, dan kontrol (susu skim-alginat) yang digunakan sebagai bahan pengkapsul. Keempat kombinasi bahan pengkapsul tersebut mempengaruhi nilai sintasan probiotik, efisiensi enkapsulasi, jumlah sel pada manik-manik basah dan manik-manik kering, jumlah sel hidup selama proses pengeringan, dan jumlah sel terenkapsulasi pada kondisi simulasi asam dan keberadaan garam empedu di lingkungan hidupnya. Berdasarkan sintasan *L. casei* dan *B. longum*, sifat matriks manik-manik, jumlah sel pada manik-manik basah dan kering, serta jumlah sel yang bertahan selama proses pengeringan, kombinasi maltodekstrin-alginat memberikan hasil yang lebih baik dibandingkan dengan kombinasi pati sagu-alginat dan pati jagung-alginat, tetapi tidak sebaik susu skim-alginat (kontrol) dalam mengenkapsulasi probiotik. Jumlah sel *L. casei* dan *B. longum* pada manik-manik kering maltodekstrin-alginat masing-masing yaitu 4.69±0.08 log CFU/g dan 5.32±0.21 log CFU/g, sedangkan jumlah sel *L. casei* dan *B. longum* pada manik-manik kering susu skim-alginat lebih tinggi yaitu masing-masing 5.08±0.07 log CFU/g dan 6.20±0.16 log CFU/g. *L. casei* lebih tahan terhadap lingkungan asam (pH rendah). Dalam keberadaan 0.3% garam empedu, *L. casei* dan *B. longum* terenkapsulasi susu skim-alginat meningkat masing-masing sebanyak 2.75±0.02 dan 1.61±0.04 siklus log.

Kata Kunci: Enkapsulasi, Ekstrusi, Probiotik, Ketahanan Sel, Sintasan

ABSTRACT

Widaningrum, Miskiyah, Indrasti D, Hidaya HC. 2018. Improvement of viability of *Lactobacillus casei* and *Bifidobacterium longum* with several encapsulating materials using extrusion method. JITV 23(4): 189-201. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1547

Lactobacillus casei and *Bifidobacterium longum* are probiotics commonly applied as dry starter for food system. Drying process in the production of dry starter can reduce the number of probiotics, therefore they are necessary to be encapsulated. Aim of this research was to obtain best encapsulating material for both probiotics. Encapsulation technique used in this research was extrusion with maltodextrine-alginate, sago starch-alginate, corn starch-alginate, and control of treatment skim milk-alginate (w:w) as encapsulating materials. The four encapsulating materials significantly affected the value of viability, encapsulation efficiency, number of cell in wet beads and dry beads, number of survival cell during drying process, and number of survival encapsulated cell in simulated acid and bile salt conditions. Based on viability of *L. casei* and *B. longum*, beads matrix characteristic, number of cell in wet beads and dry beads, and number of survival cells during drying process, maltodextrine-alginate was better than sago starch-alginate and corn starch-alginate, but was not as good as skim milk-alginate (control of treatment) as encapsulating material. Viability (number) of *L. casei* and *B. longum* in dry beads of maltodextrine-alginate were 4.69±0.08 log CFU/g and 5.32±0.21 log CFU/g, while number of *L. casei* and *B. longum* in dry beads of skim milk-alginate were higher 5.08±0.07 log CFU/g and 6.20±0.16 log CFU/g. *L. casei* more resistant than *B. longum* against acidic (low pH) environment. In the presence of 0.3% bile salt, *L. casei* and *B. longum* encapsulated with skim milk-alginate increased as much as 2.75±0. 02 and 1.61±0.04 log cycles, respectively.

Key Words: Encapsulation, Extrusion, Probiotics, Cell Resilience, Viability

INTRODUCTION

Probiotics are living microorganisms actively able to improve human health by balancing microflora in gastrointestinal tract if they are consumed in sufficient number (Fuller 1992). Probiotic consumption, in some ways, is important to maintain gastrointestinal tract (GIT) health and to improve host immune system. According to Gibson & Robertfroid (1995), enough number of bacteria in the body could improve immune system thus increase body's ability againts diseases. A number of genus of bacteria currently consumed as probiotic are *Lactobacillus, Leuconostoc, Pediococcus, Bifidobacterium, Enterococcus, Lactococcus,* and *Streptococcus* (Shah 2007), yet, the most developed probiotic come from *Lactobacillus* and *Bifidobacterium.*

Of two among species of *Lactobacillus* and *Bifidobacterium* classified as probiotic are *Lactobacillus casei* and *Bifidobacterium longum. Lactobacillus casei* or *L. casei* naturally lies on human's small intestine (Reid 1999), whereas *Bifidobacterium longum* dominantly lies on human's colon (Arboleya et al. 2016). Both bacteria are able to give health advantage for human body because they could inhibit growth of harmful bacteria and keep the balance of gastrointestinal tract (Holzapfel & Schilinger 2002).

Application of *L. casei* and *B. longum* in food generally in the form of starter of probiotic. Probiotic starter could be in the liquid or dried form. Nevertheless, usage of probiotic starter in the dried form recognized as more benefited since it easier to be used and to be packaged. In addition, dried starter could prolong the shelf life of starter (Krasaekoopt et al. 2003). Nonetheless, drying process in the production of dry starter could decrease probiotic number in the final product (starter). Thus, optimizing (and maintaining) high number of probiotic in the dry form is of fundamental importance. Increasing number of probiotic in starter can be done by encapsulating probiotic in the initial step, then dry them by the appropriate drying method. Dry starter resulted could be having probiotic bacteria in the high number. According to Tamime et al. (2006), minimum concentration of probiotic in the food product is 106 CFU/g of product.

Encapsulation is a coating process of core of a material using certain encapsulates. Core material in this case is probiotic bacteria. Purpose of encapsulation is to maintain viability of probiotic bacteria and protect them from damages caused by undesirable environment condition (Frazier & Westhoff 1998). Extrusion is encapsulation technique that done with the way of adding probiotic microorganisms into hydrocolloid of natrium alginate, then being dropped into hardening solution ($CaCl_2$) until beads containing bacteria cells (microcapsules) were being formed. Microcapsule or bead systems using various biopolymers are very easy to prepare on a lab-scale with any encapsulated ingredients. Alginate is on top among other ingredients. Nevertheless, calcium alginate beads tend to be very porous which allows fast and easy diffusion of water and other fluids in and out the matrix (microcapsules) (Anal & Singh 2007). This has to be overcome by using other potential degradable materials which may address the porosity issue. Starch is one of the biopolymers that have the potential to be investigated as encapsulating materials since it is available abundantly and relatively cheap in cost. Exploring starch as bio-encapsulating material would be worthed particularly if it is aimed to be used in the industrial scale. As extrusion technique marked as easier, cheaper, and simpler thus able to protect probiotic cells viability (Krasaekoopt et al. 2003), this technique was being chosen as encapsulating technique in this research.

Mixing of starch and alginate were used in this research. Type of starch used was maltodextrine, sago starch, and corn starch. Encapsulation of *L. casei* and *B. longum* with the mixing of starch and alginate hopefully could maintain viability of both probiotic bacteria thus dry starter of probiotic bacteria with high number of bacteria could be obtained. Besides, usage of maltodextrin, sago starch and corn starch as encapsulating material have not been developed yet, thus they can be made as new candidates for new encapsulating material. This research purposed to determine the best encapsulating material and to study the effects of encapsulating material toward viability of probiotic bacteria, encapsulation efficiency, and resilience of dry beads *L. casei* and *B. longum* against low pH and bile salt.

MATERIALS AND METHODS

Preparation of starch-alginate solution

Freeze dried isolates of probiotic bacteria *L. casei* FNCC 0090 and *B. longum* ATCC 15707 were obtained from Food and Nutrition Collection Center Gadjah Mada University Indonesia, Na-alginate, skim milk, maltodextrin, sago starch, corn starch were obtained from Yoek Shop, MRSA (de Man Rogosa Sharpe Agar, MERCK), MRSB (de Man Rogosa Sharp Broth, MERCK) for bacterial growth. Chemical used were aquadest, $CaCl_2$, NaCl, PBS, NaOH, HCl, and bile salt.

This stage initiated by preparing 20 ml of encapsulate suspension which is consist of starch-alginate with comparison 1 : 3 (1% : 3%) (w:w) from each starch, and 1 : 2 for skim milk-alginate as control of treatment. Number of total solid as much as 4%. Usage of 4% total solid refers to Mandal et al. (2006)

which reported that viability of *L. casei* NCDC 298 on low pH (1.5) had increased in line with the increase of alginate concentration. Besides, the highest viability of *L. casei* obtained from the use of 4% alginate. Skim milk-alginate as encapsulating material with composition of 1 : 2 (1.3% of skim milk : 2.7% of alginate) was used as control of treatment.

Usage of skim milk-alginate as control of treatment refers to research report of Adrianto (2011) which reported that *L. casei* encapsulated with skim milk and alginate at a ratio of 1 : 2 had higher percentage of cell resilience compared to those without skim milk addition (4% alginate) after being dried using oven on 40°C for 6 hours. Dried skim milk : alginate encapsulated-*L. casei* had 58.4% cell resilience, whereas without addition of skim milk (4% alginate) the value was just less than 22.1%. Before being used for encapsulation process, all of encapsulating material suspension was subsequently sterilized in autoclave at 121°C for 15 minutes. After cooling, the suspension was then dropped into a solution of $CaCl_2$ 0.1 M by a 10 mL syringe to form *beads* (Krasaekoopt et al. 2003). The bead was filtered using whitman filter paper and washed by sterile 0.85% NaCl.

Encapsulation of *L. casei* and *B. longum* (Krasaekoopt et al. 2003)

Encapsulation technique used was extrusion technique adopted from Krasaekoopt et al. (2003). There were four types of encapsulating materials used to encapsulate *L. casei* and *B. longum*, i.e. maltodextrine-alginate, sago starch-alginate, corn starch-alginate (with the ratio of starch to alginate was 1% : 3%) and skim milk : alginate (as control of treatment with the ratio 1% : 2%). Encapsulation process started by mixing 1% of probiotic cultures (in MRSB) into sterilized encapsulating material, then homogenized for 40 minutes. The mixture of suspension was placed into sterile syringe and dropped into 0.1 M $CaCl_2$ with the drop distance of ± 1 cm while stirring gently using magnetic stirrer at 200-350 rpm). Hardening time in 0.1 M $CaCl_2$ solution was ± 30 minutes. Subsequently, obtained beads were being screened and washed using 0.85% NaCl then being drained for 2 minutes and placed in sterile petridish. Observed parameters in this step including yields, total plate count (pouring method), encapsulation efficiency and cell viability according to method of Sheu & Marshal (1993).

Calculation of encapsulation efficiency

Methods of encapsulation process of *L. casei* and *B. longum* and way of preparation of beads for measuring total plate count based on following way: alginate was mixed with either skim milk or maltodextrin or sago starch or corn starch, then aquadest was added into the formula. The formula then being sterilized at 121°C for 15 minutes, thus cooled at ambient temperature. After that, 1% of *B. longum* and or *L. casei* were mixed each into the formula then homogenized for 40 minutes. Subsequently, the formula was being dropped into steriled 0.1 M $CaCl_2$ and drained and washed with 0.85% NaCl until beads of *B. longum* and *L. casei* were resulted. Cell number then measured, and the beads then being dried at temperature of 40°C for 7 hours. At the end of the process, dry beads of *B. longum* and *L. casei* were resulted and again the cell number was measured.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Probiotic population (CFU/g beads)}}{\text{Total probiotic in suspension (CFU/g)}} \times 100$$

Calculation of cell viability

Cell viability was calculated using equation:

$$\text{Viability (\%)} = \frac{\text{Log}\frac{\text{cfu}}{\text{G}} \text{ of wet beads}}{\text{Log}\frac{\text{cfu}}{\text{G}} \text{ of biopolymer suspension}} X\ 100$$

Calculation of yield of dried beads

Drying process aimed to dry *L. casei* and *B. longum* beads thus dry probiotic beads can be obtained. Beads drying done on 40°C using blower oven. Drying using blower oven marked as easier and cheaper compared to spray drying or freeze drying. Temperature of 40°C was used regarding the range of *L. casei* growth temperature (15-40°C) and *B. longum* (37-41°C) thus there still possibility that both probiotic bacteria still alive. Drying time was determined by measuring mass of beads during drying process until the constant masses were obtained. Observed parameters were moisture of dry beads and yield of dry beads.

$$\text{Yield of dry beads (\%)} = \frac{\text{Mass of dry beads (g)}}{\text{Mass of wet beads (g)}} \times 100$$

Calculation of cell resilience

Total plate count (pouring method), and percentage of cell resilience after drying process. Yield of dry beads and percentage of cell resilience can be counted by following formula:

$$\text{Cell resilience (\%)} = \frac{\text{Log}\frac{\text{CFU}}{\text{g}} \text{ of beads after being dried}}{\text{Log}\frac{\text{CFU}}{\text{g}} \text{ beads before being dried}}\ x\ 100$$

Resilience testing of probiotic dry beads toward low pH and bile salt (modification of Lin et al. 2006)

Resilience testing of probiotic bacteria toward low pH and bile salt aimed to study the ability of both probiotic bacteria to remain stable on gastrointestinal track environment, and to study the ability of encapsulating materials in protecting probiotic bacteria on low pH and availability of bile salt. Low pH and bile salt testing was done continually according to modification method of Lin et al. (2006). Resilience testing of probiotic bacteria toward low pH was done by adding 1 g of dry beads into 9 ml PBS (pH 2.0) arranged by using HCl 0.1 N, and then incubated for 3 hours on 37°C. After being incubated, cell number on dried beads measured by pouring method using MRSA and incubated on 37°C for 48 hours.

Resilience testing of probiotic bacteria toward bile salt was done by re-mixing (re-suspended) dry beads of bacteria after being incubated on pH 2.0 treatment into MRSB contained 0.3% of bile salt. Before being resuspended, firstly beads must be washed using PBS on pH 7.2, after that medium containing beads can be incubated on 37°C for 36 hours and the cell number was being counted (pouring method) using MRSA.

Moisture content analysis (Oven method, SNI 01-2891-1992)

Moisture content analysis was done to obtain moisture content of dry beads from each encapsulating materials. Firstly, empty cups were being dried in oven at 105°C for 15 minutes. Cups then being cooled in desiccator then being weighed (W2). Subsequently, sample were being into cups as much as 0.5 g. Dish then being dried by oven drying at 105°C for 3 hours or more until constant weight were reached. Finally, cups contained sample were being cooled in desiccator then being weighed (W2). Moisture content was measured using following formula:

$$\text{Moisture content (g/100 g dried samples)} = \frac{\text{w- (w1-w2)}}{\text{W1-w2}} \times 100$$

Statistical analysis

Statistical analysis was used to process obtained data from stages: encapsulation and drying of *L. casei* and *B. longum* beads and resilience testing of probiotic dry beads toward low pH and bile salt. Statistical analysis aimed to obtain information whether the two factors (encapsulating materials and probiotic type) had significant effect or not towards obtained data from every stage of research. Those data then processed using Analysis of Variance (ANOVA) and further testing Duncan on 95% significance level.

Experimental design

Experimental design used in this research was completely randomized design. There were two factor i.e. type of encapsulating materials (combination of maltodextrine-alginate, sago starch-alginate, corn starch-alginate, and skim milk-alginate as control of treatment) and type of probiotic (*L. casei* and *B.longum*). Data were obtained in four replications.

RESULTS AND DISCUSSION

Encapsulation of *L. casei* and *B. longum*

Viability of *L. casei* and *B. longum* can be seen on Table 1. The higher viability, the lower decrease on cell probiotic number after being encapsulated. According to statistical data (analysis of variance/anova), probiotic type and type of encapsulating material affected ($P<0.05$) viability of probiotic bacteria cells. The highest viability of *L. casei* and *B. longum* obtained from control treatment of encapsulating material (skim milk-alginate) i.e. 99.55%±0.37 on *L. casei* and 97.48%±0.22 on *B. longum*, yet, viability of both probiotic encapsulated with three others encapsulating material based on starch-alginate i.e. maltodextrine-alginate, sago starch-alginate, and corn starch-alginate also had high number which reached more than 90%. It showed there was a little decrease in cell numbers of *L. casei* and *B. longum* during encapsulation process. Jownonski et al. (1997) reported that alginate-starch capsules had ability to encapsulate *Lactobacillus acidophilus* without decreasing bacteria viability and its ability to ferment. Likewise, Adrianto (2011) reported that encapsulation of *L. casei* with encapsulating material based on protein-alginate such as skim milk, whey and sodium caseinate produced viability as much as 95% suggesting starch-alginate may have ability to encapsulate probiotic bacteria as good as material-based protein (skim milk)-alginate.

Encapsulation efficiency of *L. casei* and *B. longum* can also be seen on Table 1. According to statistical data (anova), encapsulation efficiency of *L. casei* and *B. longum* from four types of encapsulating materials were significantly different ($P<0.05$). Between the three alginate based-encapsulating materials, the lower efficiency of encapsulation obtained by sago starch-alginate (12.02%±2.10) on *L. casei* and 12.62%±2.29 on *B. longum*, whereas the higher encapsulation efficiency obtained by skim milk-alginate (control of treatment). Encapsulation efficiency was affected by yield and bacteria cell number presence on beads. The highest bacteria cell number presence on skim milk-

Table 1. Properties of *L. casei* and *B. longum* wet beads

Parameters	Skim milk-Alginate (1:2) (control of treatment)	Maltodextrine-Alginate (1:3)	Sago starch-Alginate (1:3)	Corn starch-Alginate (1:3)
Lactobacillus casei				
Cell population in encapsulating material suspensions (log CFU/g)	8.12±0.04[a]	8.25±0.13[b]	8.19±0.02[ab]	8.11±0.05[a]
Cell population in beads (log CFU/g)	8.08±0.03[a]	7.83±0.09[a]	7.50±0.07[a]	7.71±0.07[a]
Viability (%)	99.55±0.37[e]	94.81±0.49[c]	91.50±0.88[a]	95.11±0.49[c]
Encapsulation efficiency (%)	45.45±2.42[e]	22.87±2.46[c]	12.02±2.10[a]	25.61±2.40[c]
Diameter of beads (mm)	2.15±0.11	2.59±0.10	2.72±0.17	3.00±0.13
Yield of wet beads (%)	49.40±0.78[a]	61.17±0.86[c]	59.13±0.52[b]	63.64±0.58[d]
Bifidobacterium longum				
Cell population in encapsulating material suspensions (log CFU/g)	8.24±0.25[a]	8.63±0.28[b]	8.61±0.09[b]	8.36±0.06[ab]
Cell population in beads (log CFU/g)	8.03±0.22	8.06±0.26	7.94±0.05	7.94±0.09
Viability (%)	97.48±0.22[d]	93.40±0.39[b]	92.15±0.89[a]	95.02±0.44[c]
Encapsulation efficiency (%)	30.60±1.61[d]	16.59±1.45[b]	12.62±2.29[a]	24.47±1.77[c]
Diameter of beads (mm)	2.15±0.11	2.59±0.10	2.72±0.17	3.00±0.13
Yield of wet beads (%)	49.32±0.71[a]	61.43±0.58[c]	59.18±0.44[b]	63.62±0.61[d]

Value on the table were average ± standar of deviation with n=4. Value with different character showed the significant different ($p<0.05$) based on Duncan post hoc tests

alginate, therefore value of encapsulation efficiency resulted was higher than other encapsulating material. Encapsulation efficiency of *L. casei* and *B. longum* from skim milk-alginate were 45.4%±2.42 and 30.60%±1.61. Lactose content on skim milk as one of carbon source for growing lactic acid bacteria caused yield and bacteria cell number higher than other treatment.

Therefore, the percentage of the highest viability and encapsulation efficiency of *L. casei* and *B. longum* went to skim milk-alginate (control of treatment), indicating skim milk-alginate had better performance than the three others encapsulating materials. Those high percentages were caused by higher cells number of *L. casei* and *B. longum* in beads containing skim milk-alginate compared to the number in the three others encapsulating materials. These results can be explained that skim milk and alginate could form better beads matrices thus the occurrence of cell number decrease was lower and cell number can be more encapsulated compared to those with the three others encapsulating materials. Skim milk is easier to dissolve than other starch. Solubility of encapsulating materials in the preparation of suspension might a factor which affected beads matrices. Skim milk-alginate was easy to dissolve in the water-based material, whereas sago starch-alginate was the most difficult. Therefore, matrices of skim milk-alginate beads more compact compared to sago starch-alginate beads. According to Castilla et al. (2010), characteristics of beads formed inter encapsulating materials would affect the successful of encapsulation.

Viability of probiotic was affected by several factors, such as nutrient availability, strain types, presence of growth *promotor* or inhibitor, O_2 solubility, and number or inoculation level (Oliviera & Damin 2003). According to those factors, one of ways that can be done to increase number and probiotic viability on beads was to increase the level of bacteria inoculation. Increase of cell number in suspension could increase cell numbers in beads and finally it might increase viability or bacteria encapsulation efficiency (Mortazavian et al. 2007).

Drying of *L. casei* and *B. longum* Beads

Drying of *L. casei* and *B. longum* done at 40°C using blower oven. Before doing drying, optimum drying time must be determined first. Drying time was determined by measuring beads mass during drying until constant mass was reached. Curve of beads mass decrease from each of encapsulating material can be seen on Figure 1.

During drying process, there had been a decrease in beads mass. All types of encapsulating materials (skim milk-alginate, maltodextrine-alginate, sago starch-alginate, and corn starch-alginate) seem underwent weight decrease constantly across time course. In hour 5 and 6, mass beads seem got stable, yet the condition of beads from all encapsulating materials still adhere on petridish and difficult to be released. Therefore, 7 hrs drying time was chosen either on beads of: skim milk-alginate, maltodextrine-alginate, sago starch-alginate, or corn starch-alginate has reached constant mass and did not adhere to the dishes. Dry beads resulted had flat-circled and brown in color. Beads appearance after being dried can be seen on Figure 2.

Characteristics of *L. casei* and *B. longum* dry beads can be seen on Table 3. According to statistical data (anova), probiotic types did not affect moisture and yield of dry beads, because the two parameters just were affected by encapsulating materials. Encapsulation of *L. casei* and *B. longum* with maltodextrine-alginate resulted the lowest yield and moisture (4.58% with 12.50%±0.45 moisture content on *L/. casei* dry beads and 4.85% with 12.56±0.465% moisture content on *B. longum* dry beads), whereas the highest yield and moisture content resulted by encapsulating material skim milk-alginate, they were 5.16% with moisture content 11.78%±0.32 on *L. casei* dry beads and 5.29% with moisture content 11.95 ± 0.28% on *B. longum* dry beads.

Dry beads moisture content showed margin of beads before and after drying process. During drying process, water evaporation might be happened, thus decrease mass of beads would occur. Moisture content of dry beads was affected by moisture content of encapsulating material and was not affected by number of cells and bacterial cell resilience. Bacterial cell resilience much more affected by beads matrices which was formed by encapsulating material, due to beads matrices would give protection during drying process and affected cell numbers on beads after being dried.

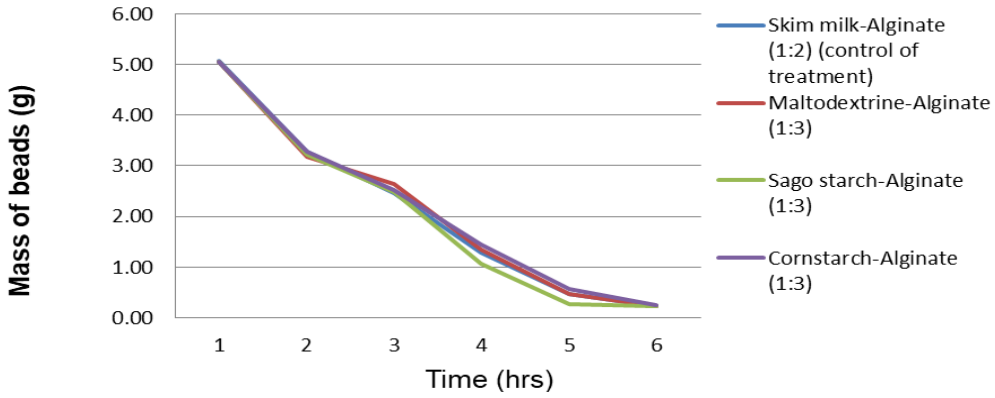
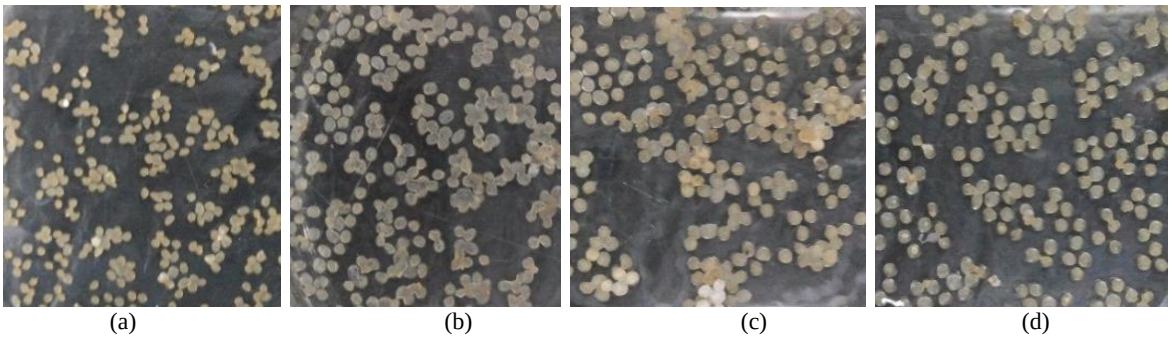


Figure 1. Curve of mass beads decrease during drying process.



(a) (b) (c) (d)

Figure 2. Appearance of dry beads made from following encapsulating materials : (a) skim milk-alginate, (b) maltodextrine-alginate, (c) sago starch-alginate, (d) corn starch-alginate.

Table 2. Properties of *L. casei* and *B. longum* dry beads

Lactobacillus casei				
Parameters	Skim milk-Alginate (1:2) (control of treatment)	Maltodextrine-Alginate (1:3)	Sago starch-Alginate (1:3)	Corn starch-Alginate (1:3)
Cell population in dry beads (log CFU/g)	5.08 ± 0.07[a]	4.69 ± 0.08[b]	3.71 ± 0.02[c]	3.49 ± 0.03[d]
Cell resilience (%)	63.84 ± 0.75[d]	59.98 ± 0.81[c]	49.56 ± 0.43[b]	45.28 ± 0.82[a]
Mass of dry beads (g)	2.55 ± 0.19	2.80 ± 0.27	3.05 ± 0.15	3.28 ± 0.13
Yield of dry beads (%)	5,16	4,85	5,16	5,15
Moisture (%)	11.78 ± 0.32[a]	12.50 ± 0.45[b]	13.25 ± 0.48[c]	13.28 ± 0.22[c]
Color	Brown	Brown	Dark brown	Brown
Bifidobacterium longum				
Parameters	Skim milk-Alginate (1:2) (control of treatment)	Maltodextrine-Alginate (1:3)	Sago starch-Alginate (1:3)	Corn starch-Alginate (1:3)
Cell population in dry beads (log CFU/g)	6.20 ± 0.16[d]	5.32 ± 0.21[b]	4.83 ± 0.09[a]	4.83 ± 0.06[a]
Cell resilience (%)	77.20 ± 0.46[f]	65.98 ± 0.70[e]	60.78 ± 0.84[c]	60.84 ± 0.87[c]
Mass of dry beads (g)	2.61 ± 0.09	2.98 ± 0.24	2.96 ± 0.22	3.16 ± 0.13
Yield of dry beads (%)	5,29	4,85	5,00	4,97
Moisture (%)	11.95 ± 0.28[a]	12.56 ± 0.46[b]	13.25 ± 0.29[c]	13.30 ± 0.24[c]
Color	Brown	Brown	Dark brown	Brown

Value on the table were average ± standar of deviation with n=4. Value with different character showed the significant different ($p<0.05$) based on Duncan post hoc tests

Value of dry yield was affected by resulted beads mass. Among four encapsulating materials, skim milk-alginate (control of treatment) had the lowest beads mass (Table 1). Yield of dry beads seems to be affected by moisture content of the beads materials, thus it led to result lowest yield than other treatment. The moisture content of maltodextrine, skim milk, sago starch, corn starch and natrium alginate were 6% (Blancard & Katz 1995), 3% (Tamime & Robinson 1989), 12-21% (Wattanachant et al. 2002),12-21% (Wattanachant et al. 2002), and 5-20% (Winarno 2008), respectively.

Probiotic population on dry beads less than those on wet beads. It is showed that during drying process, there was a decrease in bacterial cell number which presence in the beads. During drying process, *L. casei* and *B. longum* which were encapsulated with maltodextrine-alginate had lower bacteria cell decrease, compared to those with sago starch and corn starch. Encapsulation of *L. casei* with maltodextrine-alginate had decreased as much as 3.13±0.07 log cycles, whereas *B. longum* had lower decrease (2.74±0.07 log cycle). If it is compared with control of treatment, decrease on cell number of maltodextrine-alginate was still higher. Cell number of skim milk-alginate encapsulated-*L.casei* had decreased as much as 2.92±0.06 log cycles, whereas decrease of *B. longum* cell number was 1.83±0.07 log cycle for the same encapsulating material. Percentage of decrease of *B. longum* and *L. casei* cell number after being dried can be seen on Figure 3 and 4.

If both bacteria are compared, decrease of *L. casei* bacterial cell number after being dried was higher than decrease of *B. longum* on all encapsulating materials. The difference was allegedly caused by the drying temperature factor. According to Heller (2001), *L. casei* grow in the temperature range of 15-40°C with 30°C as the optimum temperature, whereas *B. longum* could grow in the temperature range of 37-41°C with 37°C as optimum temperature (Holt et al. 1994). Temperature used in the drying process of *L. casei* and *B. longum* beads was 40°C. Margin of drying temperature and optimum temperature of *B. longum* growth was lower than those on *L. casei.* Therefore, death cell number of *B. longum* during drying process was lower than *L. casei*.

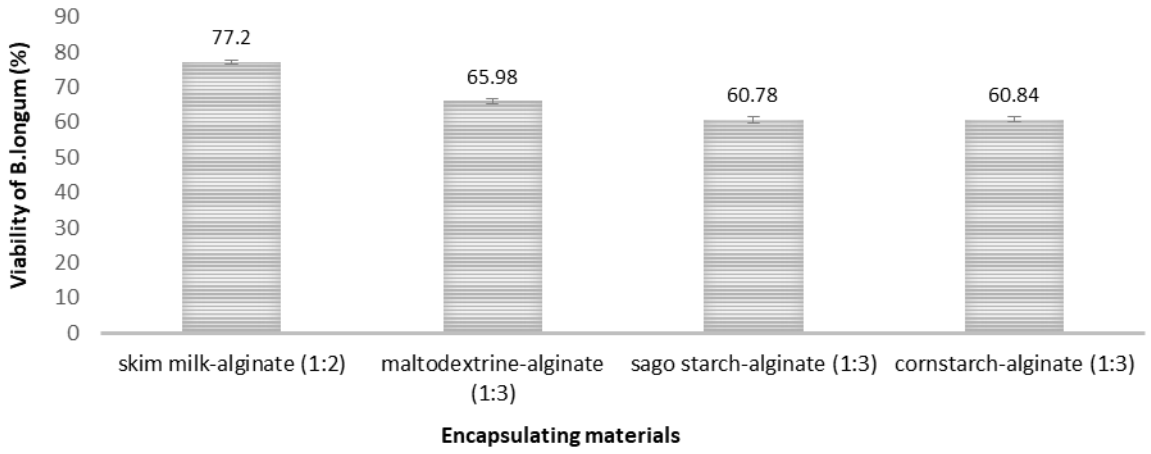


Figure 3. End population of *B. longum* after drying process (let the initial population in the wet beads = 100%).

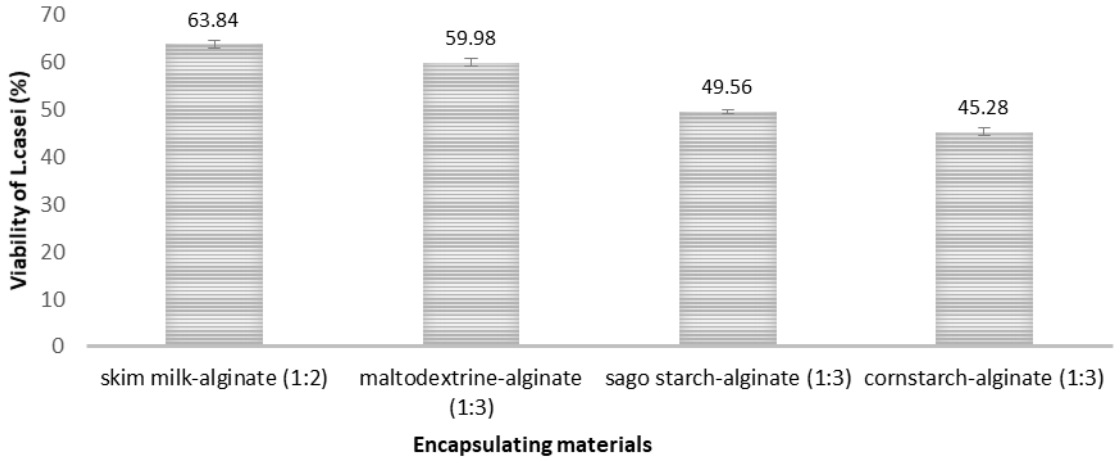


Figure 4. End population of *L. casei* after drying process (let the initial population in the wet beads = 100%).

Aside from temperature factor, decrease in *L. casei* and *B. longum* cell numbers during drying process might be caused by the loss of free water which act as important component of cells as well as the availability of oxygen. Free water is needed by bacteria to be used in metabolism process such as to synthesize cellular components, to help transport of nutrition, and to mediate other biochemical reactions (Rahayu & Nurwitri 2012). Therefore, decrease in free water number during drying process could decrease bacterial cell number. Oxygen is a sort of poison for lactic acid bacteria which may lead damages in bacterial membrane (Talwakar & Kailasapathy 2004). As long as drying process, beads were exposure to oxygen comes from air that presence in the oven, if oxygen reacts with bacterial cell it would lead oxidation and cell death.

Probiotic drying process either by oven, spray drying or freeze drying would lead decrease in bacterial cell number. According to Texeria et al., (1995), the loss of cell viability during spray drying was related to damages of cell components, membrane cell, cell walls and bacterial DNA because of high temperature that was used. Meanwhile on freeze drying, the presence of cell and medium cooling step to reach freezing point, forming of intra and extracellular ice, thawing process and reducing water in drying process leading the bacterial cell number decrease (Johnson & Etzel 1995). Whereas on drying process using oven, the main cause of decrease in bacterial cell number during drying process were temperature, water loss and the presence of oxygen.

The value of bacterial cell number decrease after being dried affected toward percentage of cell bacterial resilience. The higher the decrease of cell number, the lower its resilience. According to statistical data (anova) probiotic types and encapsulating material types significantly affected ($P<0.05$) the percentage of cell resilience after being dried. The values of *L. casei* and *B. longum* resilience from each encapsulating materials can be seen on Table 3.

Among of all starch based-encapsulating materials used in this research, percentage of the highest cell resilience was obtained from *L. casei* and *B. longum* encapsulation with maltodextrine-alginate which reached 59.98%±0.81 and 65.98%±0.70. Nevertheless, if it is compared with control of treatment, percentage of cell resilience resulted by skim milk-alginate was still higher (63.84±0.75 and 77.20%±0.46). According to Lian et al. (2003), protein-based material such as skim milk was marked better as wall in protecting cell from heat (act as thermo protectant) compared to gelatin, soluble starch, and gum arab thus it was common to be used in drying process.

Castilla et al. (2010) reported that beads characteristics formed inter encapsulating materials affected the successful of probiotic encapsulation. The more compact beads matrices formed inter encapsulating material, the more it gave protecting toward probiotic cells. Beads compactness was affected by encapsulating material solubility when suspension was being made. Among three starch based-encapsulating materials that used, maltodextrine was the most soluble material with alginate and water compared to sago and corn starch. Although sago starch and corn starch can be dissolved after sterilization process, but clumps of solids still can be found in the suspension suggesting the starch did not dissolve completely in alginate, different from maltodextrine that could dissolve completely in alginate. According to Blancard & Katz (1995), maltodextrine had high soluble properties and strong bond power. Similar to maltodextrine, skim milk also could well dissolve when it was mixed with water and alginate. The good solubility of encapsulating material would result more compact beads because maltodextrine and skim milk will fill the porous spaces formed at natrium alginate matrices. Those will reduce direct contact between cell and outer environment for example with air (oxygen) and heat during drying process. Therefore, encapsulation of *L. casei* and *B. longum* with skim milk-alginate and maltodextrine-alginate resulted higher cell resilience compared to sago starch-alginate and corn starch-alginate.

According to the percentage of cell resilience and decrease of cell numbers during drying process, encapsulation of *L. casei* and *B. longum* with maltodextrine-alginate was better than sago starch-alginate and corn starch-alginate, nonetheless, it had not better yet than those which was encapsulated with skim milk-alginate (control of treatment). Cell number of *L. casei* and *B. longum* on dry beads from encapsulating material maltodextrine-alginate was 4.69±0.08 and 5.32±0.21 log CFU/g. This number was fewer than those encapsulated with skim milk-alginate (control of treatment) which the number reached 5.08±0.07 and 6.20±0.16 log CFU/g. According to Tamime et al. (2005), minimum concentration of probiotic on food products was 10^6 CFU/g. Referring to that, dry beads from skim milk-alginate encapsulated *B. longum* seems fulfill the requirement of minimum probiotic concentration. Aim of encapsulation in this research was to produce probiotic dry starter. On its application, starter will be added to milk, then during fermentation time it will be increase in bacterial cell number which was generally will be followed with its increasing viscosity and milk acidity. Adrianto (2011) reported that application dry encapsulated *L. casei* as starter for cow dadih led to the increase of viscosity and milk acidity and the increase of *L. casei* cell number as much as 3 log cycles after 48 hours fermentation time.

Resilience testing of probiotic dry beads against low pH and bile salt

This test aimed to study ability of both probiotic bacteria to survive and their endurance and persistency in gastrointestinal environment, and to study ability of encapsulating materials in protecting both probiotic bacteria in low pH condition and the presence of bile salt. According to Schmid et al. (2006), resilience testing againts low pH and bile salt can be done to test matrices of certain material in protecting probiotic cells. Gastrointestinal is main place which can affect probiotic bacteria viability in the human body. Gastrointestinal track started from mouth, esophagus, gastric, small intestine, colon, and end up in rectum. Gastric and small intestine is a critical location when in these places occur pH reduction and bile salt secretion. Therefore, to get small intestine, probiotic must be able to stay alive at low pH and in the presence of bile salt (Sahadeva et al. 2011).

Testing the resilience of *L. casei* and *B. longum* against low pH and the presence of bile salts was carried out continuously. According to the results, incubation of dry beads from the four encapsulating materials on PBS at pH 2.0 led the decrease of *L. casei* and *B. longum* bacterial cells. Decrease in *L. casei* and *B. longum* bacterial cells was significantly different ($P<0.05$) on each encapsulating materials. Decrease of *L. casei* and *B. longum* from encapsulating material skim milk-alginate (control of treatment) was 0.19±0.08 and 0.93±0.18 log cycles. The decrease was lower than those of *L. casei* and *B. longum* encapsulated with the

three others encapsulating materials starch-alginate. Graph of decrease of the number of *L. casei* and *B. longum* led by treatment of pH can be seen on Figures 5 and 6.

Decrease in *L. casei* and *B. longum* during incubation process was caused by dissociated of HCl (hydrochloric acid) that resulted proton which led pH decrease in the outer cell media (extracellular pH). In a very acidic condition such as pH 2.0, pH of cell cytoplasmic (intracellular pH) could decrease, and the damages of bacterial cell outer membrane could occur leading the death of cell (Hutkins & Nannen 1993). Degree of cell resilience against low pH was varying on each bacteria. Based on decrease of *L. casei* and *B. longum* cell number after being incubated at pH 2.0, it is known that decrease of *L. casei* cell number from all types of encapsulating materials was lower than those on *B. longum.* It is shown that genus of *Lactobacillus* more resistant against acidic condition rather than *Bifidobacterium.* Widodo (2003) reported that genus of *Lactobacillus* could grow at the range of pH 3.5-6.8, whereas genus of *Bifidobacterium* could grow at the narrower pH range (5.5-7.0). Bacterial tolerance against low pH due to their bacterial could maintain intracellular pH (cytoplasmic pH) to be stable at alkaline pH against extracellular pH. Although, decrease in intracellular pH will still keep continuing in agreement with the decrease of extracellular pH. Therefore, although probiotic genus like *Lactobacillus* tolerant to acidic condition, the decrease of cell numbers would still happen.

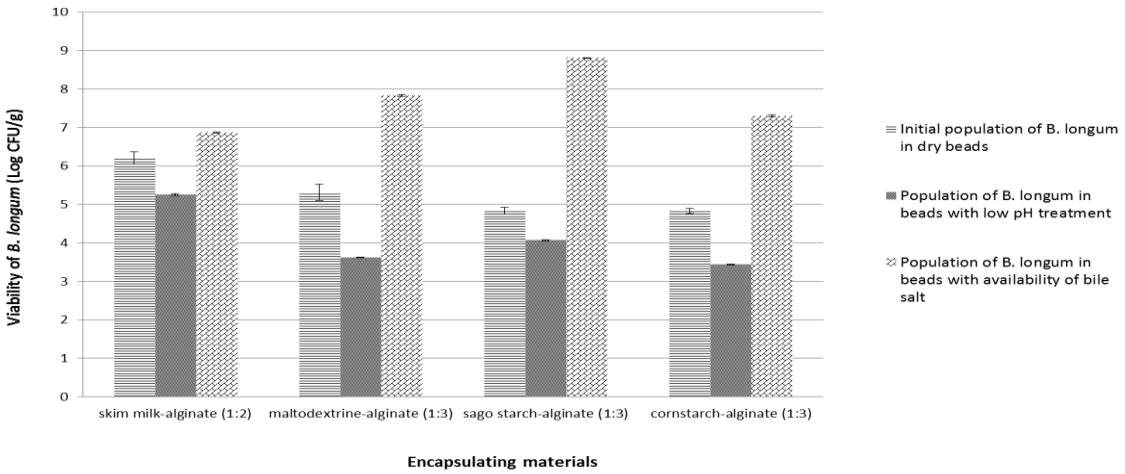


Figure 5. Graph changes on cell bacterial number (*B. longum*) due to low pH treatment and availability of bile salt.

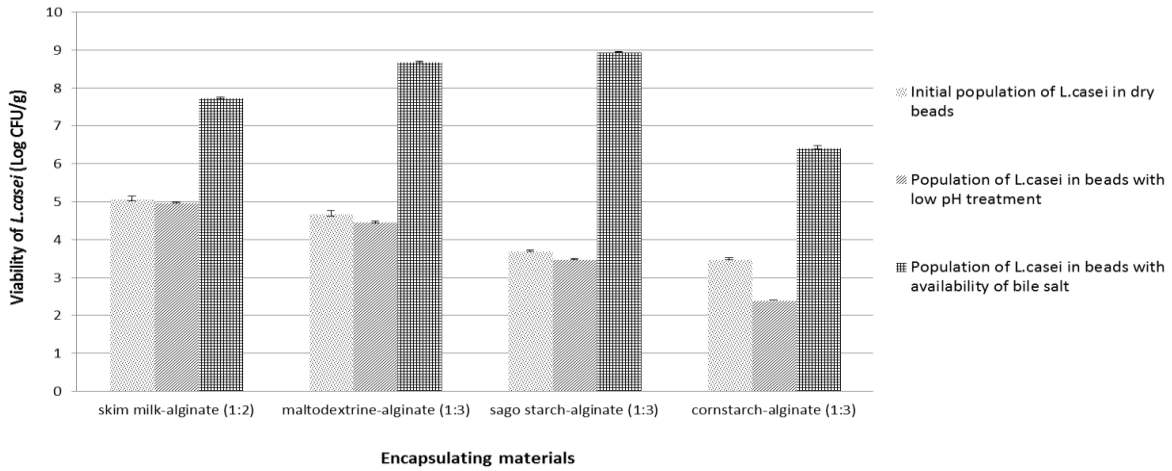


Figure 6. Graph changes on cell bacterial number (*L. casei*) due to low pH treatment and availability of bile salt.

Sultana et al. (2000) reported that testing the resilience of Hi-maize resistant starch encapsulated-*L. acidophilus* and *B. infantis* against low pH led the decrease of cell numbers as much as 5 and 3 log cycles after being incubated for 3 hours at pH 2.0. This was also happened on this research, compared to bacterial cell number on dry beads, cell number on beads which were incubated at the same condition also decreased. The decrease of *L. casei* and *B. longum* cell number was fewer than 2 log cycles. If it is compared with research results of Sultana et al. (2000), the decrease of bacterial cells caused by low pH in this research was lower. It is shown that encapsulation of *L. casei* and *B. longum* with maltodextrine-alginate, sago starch-alginate, corn starch-alginate, and skim milk-alginate (control of treatment) could protect bacterial cells on low pH.

Testing resilience of *L. casei* and *B. longum* dry beads against low pH and bile salt was carried out continuously. After being incubated for 36 hours on MRSB contained 0.3% of bile salt, there was an increase in the cell number of *L. casei* and *B. longum* compared to bacterial cell number that could survive at the low pH. The bacterial cell increase can be seen on Figure 8. Statistically, the increase of bacterial cell number after bile salt treatment was significantly different ($P<0.05$) on each encapsulating materials. After incubation process, cell number of *L. casei* from encapsulating material maltodextrine-alginate, sago starch-alginate, and corn starch-alginate increased successively as much as 4.22±0.03, 5.47±0.02 and 4.03±0.04 log cycles, whereas *B. longum* cell number were 4.21±0.03, 4.74±0.02, and 3.87±0.01 log cycles for the consecutive encapsulating materials. If compared with these three types of encapsulating materials, increase of cell number of *L. casei* and *B. longum* with encapsulating materials skim milk-alginate (control) was lower. Cell number of *L. casei* and *B. longum* from encapsulating material skim milk-alginate had increased 2.75±0.02 and 1.61±0.04 log cycles after 36 hrs incubation.

Jacobsen et al. (1999) reported that 0.3% bile salt was representative to test bacterial resilience against bile salt. Bacterial incubation in media containing bile salt generally would reduce bacterial cell number. This is caused by ability of bile salt to reduce lipid which was substance that arrange bacterial cell membranes. As a result, damages occurred on the bacterial cell membranes followed by leakages and cell lysis (Hill 1995). Different from testing of *L. casei* and *B. longum* dry beads against bile salt in this research, incubation of *L. casei* and *B. longum* in MRSB containing bile salt may have increased the bacterial cell number. This increase highly likely caused by the resilience of both bacteria against bile salt and use MRSB which has function as source of nutrition and medium of growth. According to Djide & Wahyudin (2008), lactic acid bacterial isolates are able to grow in the medium, although bile salt has been added into the medium. Lin et al. (2006) also showed that there was increase some types of lactic acid bacterial as much as 1-4 log cycles after being incubated in MRSB containing 03% bile salt for 36 hours. Besides using MRSB, some resilience testing against bile salt also use sterile solution which was mixed with bile salt (Castilla et al. 2010; Lee & Heo 2000). Usage of those sterile solutions can be done to avoid increase of bacterial cell number which might be caused by the availability of nutrition from media.

Increase of *L. casei* and *B. longum* bacterial cell number after being incubated from encapsulating materials based on starch (maltodextrine-alginate, sago starch-alginate, and corn starch-alginate) was higher than those with skim milk-alginate (control of treatment). This was possible due to matrices of encapsulating materials. On encapsulating materials maltodextrine-alginate, sago starch-alginate, and corn starch-alginate, higher alginate concentration (3%) compared with skim milk-alginate (2.67%). Skim milk-alginate composition used in this research was the best result from previous study, while for the treatment were from previous optimation steps, thus presumably led beads from those encapsulating materials become porous.

According to Rokka & Rantamäki (2010), matrices of alginate were very porous so as to cause water diffusion in and out of beads. During incubation time, MRSB diffused into maltodextrine-alginate beads, sago starch-alginate beads, corn starch-alginate beads, and skim milk-alginate beads thus cause bacterial colonization. The highest increase of *L. casei* and *B. longum* cell number went to sago starch-alginate, whereas to skim milk-alginate (control of treatment), the increase of *L. casei* and *B. longum* were the lowest. Meanwhile on skim milk-alginate, it was allegedly that skim milk could fill more much alginate pores thus resulted in more solid beads matrices and formed barrier of media diffusion into beads. According to Castilla et al. (2010), solidity of encapsulating material matrics would affect ability of material to absorb and protect bacteria from acid and bile salt effects. Although more solid beads matrices could increase of lower bacterial cell number, dry beads with compact encapsulating material matrices hopefully could more protect probiotic from outer environment during handling and storage (Frazier & Westhoff 1998).

CONCLUSION

Maltodextrine-alginate was the best treatment compared to other encapsulating material (sago starch and corn starch) to viability, beads matrices characteristic, cell number on wet and dry beads, and

percentage of resilience cell of *L. casei* and *B. longum* during drying process.

Cell number of maltodextrine-alginate–encapsulated *L. casei* and *B .longum* in the form of dry beads was 4.69±0.08 log CFU/g and 5.32±0.21 log CFU/g, whereas cell number of skim milk-alginate–encapsulated *L. casei* and *B. longum* in the form of dry beads were higher, they are 5.08±0.07 log CFU/g and 6.20±0.16 log CFU/g.

Resilience of both encapsulated probiotics was different against low pH and availability of bile salt, whereas *L. casei* more resistant than *B. longum* against acidic (low pH) environment due to its tolerance to a wider range of pH compared to *B. longum.*

In the presence of 0.3% bile salt, *L. casei* and *B. longum* encapsulated with skim milk-alginate increased as much as 2.75±0.02 and 1.61±0.04 log cycles, respectively.

REFERENCES

Adrianto A. 2011. Enkapsulasi *Lactobacillus casei* dengan teknik ekstrusi sebagai *starter* untuk pembuatan dadih susu sapi [Script]. [Bogor (Indones)]: Bogor Agricultural University.

Anal A, Singh H. 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. J Trends Food Sci Technol. 18:240-251.

Arboleya S, Watkins C, Stanton C, Ross P. 2016. Gut biifidobacteria populations in human health and aging. Front Microbiol. 7:1-9.

Blancard PH, FR Katz. 1995. Starch hydrolisis in food polysaccharides and their application. New York (USA): Marcell Dekker.

Castilla OS, Calleros CL, Galindo HSG, Ramirez JA, Carter EJV. 2010. Textural properties of alginate-pectin *beads* and survivability of entrapped *Lb. casei* in simulated gastrointestinal condition and in yoghurt. J Food Res Int. 43:111-117.

Djide MN, Wahyudin E. 2008. Isolasi bakteri asam laktat dari air susu ibu dan potensinya dalam penurunan kadar kolesterol secara in vitro. Majalah Farmasi dan Farmakologi. 12:73-78.

Frazier WC, Westhoff DC. 1998. Food microbiology. 4th ed. Singapore (Singapore): McGraw-Hill Book Co.

Fuller R. 1992. Probiotics: The scientific basis. New York (USA): Chapman & Hall.

Gibson GR, Robertfroid MB. 1995. Dietary modulation of the human colonic microbiota-Introducing the concept of prebiotics. J Nutr. 125:1401-1412.

Heller KJ. 2001. Probiotic bacteria in fermented foods: Product characteristics and starter organisms. Am J Clin Nutr. **7**3:374-379.

Hill MJ. 1995. Role of gut bacteria in human toxicology and pharmacology. New York (USA): Taylor & Francis.

Holt JG, Krieg NR, Sneath PHA, Staley JL, Williams ST. 1994. Bergey's manual of determinative bacteriology. 9th ed. Baltimore (USA): Williams and Wilkins Co.

Holzapfel WH, Schillinger U. 2002. Introduction to pre- and probiotics. J Food Research Int. 35:109-116.

Hutkins EW, Nannen NL. 1993. pH homeostasis in lactic acid bacteria. J Dairy Sci. 76:2354-2365.

Jacobsen CN, Nielsen VR, Hayford AE, Moller PL, Michaelsen KF, Erregaard AP, Sandstorm B, Tvede M, Jacobsen M. 1999. Screening of probiotic activities of forty seven strains of *Lactobacillus* spp. By in vitro techniques and evaluation of the colonization ability of five selected strains in human. J Appl Enviroment Microbiol. 65:4949-4956.

Johnson JAC, MR Etzel. 1995. Properties of *Lactobacillus helveticus* CNRZ-32 attenuated by spray drying, freeze drying of freezing. J Dairy Sci. 78:761-768.

Jownonski T, Zialinska M, Wyskakoska A. 1997. Encapsulation of lactic acid bacteria with alginate/strach capsule. J Biotechnol Techn. 13:3-13.

Krasaekoopt WB, Bhandari H, Deeth H. 2003. Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J. 13:3-13.

Lee K, Heo T. 2000. Survival of *Bifidobacterium longum* immobilized in calcium alginate *beads* in simulated gastric juices and bile salt solution. J Appl Environment Microbiol. 66:869-873.

Lian WC, Hsiao HC, Chou CC. 2003. Viability of microencapsulated bifidobacteria in simulated gastric juice and bile solution. Int J Food Microbiol. 86:293-301.

Lin WH, Hwang CF, Chen LW, Tsen HY. 2006. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. J Food Microbiol. 23:78-81.

Mandal S, Puniya AK, Singh K. 2006. Effect of alginate concentrations on microencapsulated *Lactobacillus casei* NCDC-298. Int Dairy J. 16:1190-1195.

Mortazavian A, Razavi SH, Ehsani RM, Sohrabvandi S. 2007. Principles and methods of microencapsulation of probiotic microorganisms. Iranian J Biotechnol. 5:1-18.

Oliviera MN, Damin MR. 2003. Efeito do teor de solidos e da concentrcao de sacarosee na acidificaca, fermeza e viabilidade de bacterias do iogurte e probioticas em leite fermentado. Ciencia e Technologia de Alimentos. 23:172-176.

Rahayu WP, Nurwitri CC. 2012. Mikrobiologi pangan. Bogor (Indones): IPB Press.

Reid G. 1999. The scientific basis for probiotic strains of Lactobacillus. Appl Environment Microbiol. 65:3763-3766.

Rokka S, Rantamäki P. 2010. Protecting probiotic bacteria by microencapsulation: Challenges for industrial application. European J Food Res Technol. 231:1-12.

Sahadeva RPK, Leong SF, Chua KH, Tan CH, Chan HY, Tong EV, Wong SYW, Chan HK. 2011. Survival of commercial probiotic strains to pH and bile. Int Food Res J. 18:1515-1522.

Schmid K, Schlothauer RC, Friedrich U, Staudt C, Apajalahti J, Hansen EB. 2006. Development of probiotic food ingridient. In: Goktepe I, Junena VK, Ahmedna M, editors. Probiotics in food safety and human health. New York (USA): CRC Press.

Shah NP. 2007. Functional culture and health benefits. Int Dairy J. 17:1262-1277.

Sheu TY, Marshall RT. 1993. Microentrapment of *lactobacilli* in calcium alginate gels. J Food Sci. 58:557-561.

SNI 01-2891-1992. Jakarta (Indones): Badan Standarisasi Nasional.

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K. 2000. Encapsulation of probiotics bacteria with alginate starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int J Food Microbiol. 62:47-55.

Talwakar A, Kailasapathy K. 2004. The role of oxygen in the viability of probiotic bacteria with reference to *L. acidophilus* and *Bifidobacterium* spp. J Current Issues Intestinal Microbiol. 5:1-8.

Tamime AY, Saarela M, Sondergaard AK, Mistry VV, Shah NP. 2006. Production and maintenance of viability of probiotic micro-organism in dairy product. In: Tamime AY, editor. Probiotic dairy products. Oxford (UK): Blackwell Publishing.

Tamime AY, Robinson AK. 1989. Yoghurt science and technology. Oxford (UK): Pergamon Press Ltd.

Texeria PC, MH Castro, FX Malcata, RM Kirby. 1995. Survival of *Lactobacillus delburgi ssp. Bulgaricus* following dray drying. J Dairy Sci. 78:1025-1031.

Wattanachant S, Muhammad SKS, Hashim DM, Rahman RA. 2002. Suitability of Sago Starch as a Base for Dual-Modification. J Sci Technol. 24:431-438.

Widodo. 2003. Bioteknologi industri susu. Cetakan Pertama. Yogyakarta (Indones): Lacticia Press.

Winarno FG. 2008. Kimia pangan dan gizi. Edisi Terbaru. Bogor (Indones): M-Brio Press.

Effect of Electrical Stimulation on Physical and Organoleptic Properties of Muscovy Duck Meat

Hafid H[1], Napirah A[1], Sarifu SM[1], Rahman[1], Inderawati[1], Nuraini[1], Hasnudi[2]

[1]*Department of Animal Science, Universitas Halu Oleo*
Jl. HEA. Mokodompit Kampus Anduonohu 93232 Kendari
[2]*Departement of Animal Husbandry, Faculty of Animal Husbandry,*
Universitas Sumatera Utara, Kampus Padang Bulan 20155 Medan
harapinhafid14@gmail.com

(received 17-09-2018; revised 06-12-2018; accepted 12-12-2018)

ABSTRAK

Hafid H, Napirah A, Sarifu SM, Rahman, Inderawati, Nuraini, Hasnudi. 2018. Pengaruh stimulasi listrik pada sifat fisik dan organoleptik daging itik Muscovy. JITV 23(4): 202-209. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1914

Penelitian ini bertujuan untuk mempelajari pengaruh lama stimulasi listrik terhadap sifat fisik dan organoleptik daging itik *Muscovy*. Penelitian ini menggunakan 20 ekor itik *Muscovy* betina, umur 1,5-2 tahun. Itik dibagi menjadi 5 kelompok perlakuan untuk 4 kali ulangan. Perlakuan adalah lama stimulasi listrik: 0, 5, 10, 15 dan 20 menit. Hasil penelitian menunjukkan bahwa lama stimulasi listrik tidak mempengaruhi (P>0,05) susut masak tetapi secara signifikan mempengaruhi (P<0,05) keempukan, warna, rasa, aroma, pH dan *juiciness* daging itik *Muscovy*. Perlakuan terbaik adalah lama stimulasi 20 menit.

Kata Kunci: Daging Itik *Muscovy*, Stimulasi Listrik, Sifat Fisik, Sifat Organoleptik

ABSTRACT

Hafid H, Napirah A, Sarifu SM, Rahman, Inderawati, Nuraini, Hasnudi. 2018. Effect of electrical stimulation on physical and organoleptic properties of Muscovy duck meat. JITV 23(4): 202-209. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1914

This research was aimed to study the effect of electrical stimulation period on physical and organoleptic properties of Muscovy duck meat. This research used 20 female Muscovy ducks, 1.5-2 years of age. The ducks were divided into 5 groups treatments for 4 replications. The treatments were period of electrical stimulation: 0, 5, 10, 15, and 20 minutes. The result showed that period of electrical stimulation did not affect (P>0.05) cooking loss but significantly affected (P<0.05) the tenderness, color, flavour, aroma, pH, and juiciness of duck meat. The best treatment was 20 minutes stimulation.

Key Words: Muscovy Duck Meat, Electrical Stimulation, Physical Properties, Organoleptic Properties

INTRODUCTION

Muscovy duck is one of poultry family that has been developed for both meat and eggs producers. In term of human nutritional, the quality of Muscovy duck meat is almost similar to beef, chicken, sheep, and goats. Muscovy duck meat has a high nutritional value, cheaper price and lower fat. Even so, the duck meat is less attractive because of rough meat fiber (Randa et al. 2002; Hafid et al. 2015). Physical quality of muscovy duck was affected by ante mortem and post-mortem factors, such as species, sex, age, muscle location, preserving methods, period and temperature of storage, packaging, and others treatments (Soeparno 2009; Hafid 2011).

Tenderness is the most important palatability factor that affect consumer acceptance against poultry meat. The older the age, the lower the tenderness of meat. The tenderness process occurred during the change of meat's physical and chemical compound, such as rigormortis process which was related to muscle ATP content. The depletion of ATP will cause the overtopping between actin miofilamin and myosin miofilamen. Then, they will locked together to form a permanent bond, actomyosin, that cause muslces became rigid and cannot be moved. This is the cause of meat became hard (Hafid et al. 2017; Soeparno 2009).

Electrical stimulation is one of the ways to improve the meat tenderness. Electrical stimulation will accelerate the post-mortem glycolysis that occurs during conversion muscle into meat, so that speed up the decline in pH as well as speeding up the release of the protease enzyme (Hafid et al. 2014). Electrical stimulation can change the characteristics of the palatability of meat and has been proven to decrease the post-mortem pH, improve tenderness, increase the post-mortem glycolysis rate, and prevent muscle shortening due to cold temperatures (Aberle et al. 2001). Hafid et al. (2014) and Hafid & Syam (2012) reported that applying 20 volt electrical stimulation during 2 minutes could increase the tenderness, color, and texture of duck meat

Researches on electrical stimulation on animal meat had been done either on large animal or poultry, but not on Muscovy duck. Especially from traditional rearing

patterns ranging in age from 1.5 to 2 years. The aim of this research was to study the effect of electrical stimulation on physical and organoleptic properties of Muscovy duck. The result of this research was expected to increase the palatability of Muscovy duck. In particular, in order to change people's perceptions of Muscovy ducks as inferior birds whose flesh smelled rancid and tough. Thus Muscovy duck farming will be developed more because it is an alternative raw material for the culinary industry in the future.

MATERIALS AND METHODS

Time and place

This research taken place in Animal Production Science and Technology Laboratory, Faculty of Animal Science, Universitas Halu Oleo, Kendari.

Material

The study used 20 female Muscovy ducks, 1.5-2 years of age. The muscovy ducks were obtained from breeder in Konda, South Konawe Sub District. Dual Tracking Supply electrical stimulator in the form of an adapter with electricity as energy source, knives, forks, plates, water bath, polyethylene plastic, pH meter, tissue, glass, bowl, and stationery were used.

Research procedure

This research was conducted with 2 phases such as material preparation, and samples preparation. Electrical stimulation was given by creating an electrical flow in certain voltage (20 volt) and certain period of stimulation. The positive pole of electric stimulator was set on Muscovy duck neck, and the negative pole was set on Muscovy duck shank.

The Muscovy ducks were slaughtered according to Islamic sharia, which is cut on the artery, jugular venous, and throat then separated between carcass and non-carcass parts. Immediately after the cuts without waiting for rigormortis process, the carcass samples were collected. The carcass samples were taken from breast meat, and then given electrical stimulation. Then, water and water bath were prepared for boiling process.

All treatments were applied systematically and repeated 4 times. Samples were placed in polyethylene plastic and labelled for organoleptic test. The given label was folded lengthwise. The plastic was folded to prevent direct contact with water during boiling process, according to the instructions of Soekarto & Hubeis (1992).

Samples were boiled in a water bath with a temperature of 80°C for 45 minutes. Water bath was used to get a stable heating temperature during boiling process. Then, the samples were taken and cooled at room temperature. If there was liquid on the meat surface, it can be dried using suction paper (Hafid et al. 2014). For organoleptic test, samples were cut to the size of 1x1 cm. Organoleptic test used 10-15 semi trained panelist according to instructions in Soekarto & Hubeis (1992). Organoleptic assessment used an assesment scale from 1 to 5 as described in Table 1

Experimental design

This research used a completely randomized design (Gasperz 2010) with 5 treatments and 4 replications. The treatments were 20 volt electrical stimulation in various period : 0 minutes (control), 5 minutes, 10 minutes, 15 minutes, and 20 minutes.

Table 1. Hedonically scale on organoleptic test

Variables	Hedonical Scale	Criteria
Tenderness	1	Very tender
	2	Tender
	3	Medium
	4	Hard
	5	Very hard
Color	1	White
	2	Pale white
	3	Pinkish
	4	Bright red
	5	Dark red
Texture	1	Highly preferred
	2	Preferred
	3	Enough preferred
	4	Not preferred
	5	Highly not preferred
Flavour	1	Highly preferred
	2	Preferred
	3	Enough preferred
	4	Not preferred
	5	Highly not preferred
Juiciness	1	Very Juicy
	2	Juicy
	3	Juicy enough
	4	Rather juicy
	5	Dry
Texture	1	Very smooth
	2	Smooth
	3	Medium
	4	Rough
	5	Very rough

(Hafid & Syam 2012); (Hafid et al. 2015)

P0 = Without electrical stimulation
P1 = 5 minutes electrical stimulation
P2 = 10 minutes electrical stimulation
P3 = 15 minutes electrical stimulation
P4 = 20 minutes electrical stimulation

The used formulation was described as follow:

$Y_{ijk} = \mu + A_{i} + \varepsilon_{ij}$

While : Y_{ijk} = Value of observed variable
μ = Mean
A_i = Effect of electrical stimulation
ε_{ij} = Error

Measured variables

The measured variables were:

1. Physical Properties, consisted of:
   a. Meat pH, was measured using a digital pH meter.
      Meat pH test was done using Bouton et al. (1971) methods as described by Soeparno (2009). Ten grams of meat samples were mashed and mixed with 10 ml of aquadest then stirred until homogenized. pH meter then cleaned using aquadest and put into a 7 pH of buffer for calibration. Each meat solution was measured its pH three times and the results were averaged.
   b. Cooking loss.
      The cooking loss measuring was done following Syam et al. (2013) methods. The meat was boiled in a temperature of 80°C during 45 minutes, cooled on room temperature, and then cooled on lower temperature ± 0°C. The samples then dried up using tissue paper. Samples then weighed to obtain its weight.
2. Organoleptic Properties
   a. The color of boiled meat was determined following instruction of Hafid et al. (2014) and Hafid & Syam (2012). The color was classified into 5 categories: white, pale white, pink, bright red and dark red.
   b. Meat tenderness was determined based on Hafid et al. (2014) instructions. The tenderness was classified into 5 categories: very tender, tender, medium, hard, and very hard.
   c. Flavour (level of deliciousness) was determined following Hafid et al. (2014) instructions which was classified from highly preferred to highly not preferred.
   d. Texture was determined following Hafid et al. (2014) instruction which was classified into 5 categories: very smooth, smooth, medium, rough, and very rough.
   e. Juiciness was determined following (Hafid et al. 2014) instruction which was classified into 5 categories: very juicy, juicy, juicy enough, dry, very dry.

Data analysis

The obtained data were analyzed using analysis of variance and continued using least significant different test based on Gasperz (2010).

RESULTS AND DISCUSSION

pH

The average of Muscovy duck meat pH were presented in Table 2.

Result of variance analysis showed that stimulation period did not affect (P>0.05) Muscovy duck meat pH. This result was similar with Lukman (2010) who explained that the pH value of meat will never reach under 5.3. This was caused by the enzymes involved in anaerobic glycolysis, which was not actively working. Likewise with Khasrad et al. (2018) who reported that the duration of electrical stimulation did not affect the pH of meat.

According to Buckle et al. (1987) and Garcia (2009), the reached final pH has a major influence on the meat quality. The higher pH caused meat to have a closed structure or solid with a dark purple color, less tasty, and more condition that allow the development of microorganisms.

The decline in the value of post-mortem muscle pH was also determined by the rate of post-mortem glycolysis and reserves of meat glycogen, normally was 5.4 up to 5.8 (Soeparno 2009). (Lee et al. 2000) explained that a stressed animal would have a lower glycogen reserves and ATP so that the animal energy would deplete shortly after died and the level of Ca^{2+} in the sarcoplasmic would quickly increase. High level of Ca^{2+} will trigger the overhaul of glycogen in a short time, so rigormortis will occur faster while pH remains high.

Cooking loss

Cooking loss is a heavy percentage of meat lost due to cooking and is a function of cooking time and temperature. Meat with a low cooking shrinkage has a relatively better quality than meat with a high percentage of cooking losses, this is because the loss of nutrients during the cooking process will be less (Komariah et al. 2009). Cooked meat is an indicator that shows the freshness of meat, where new meat is finished slaughtering will have a low cooking loos.The average of cooking loss of Muscovy duck meat stimulated with electricity, were presented in Table 3.

Result of the study showed that electrical stimulation period did not give a significant effect (P>0.05) on cooking loss of Muscovy duck meat. In this

Table 2. The average of pH of muscovy duck meat stimulated electrically with different period

Replication	Electrical stimulation period (minutes)				
	Control	5	10	15	20
I	5.75	5.53	5.39	5.66	5.59
II	5.75	5.51	5.61	5.91	5.61
III	5.83	5.77	5.95	6	6.62
IV	6.04	5.56	5.31	6.09	5.94
Average	5.84±0.14	5.59±0.12	5.57±0.29	5.92±0.19	5.94±0.48

Table 3. The average of cooking loss of muscovy duck meat stimulated electrically with different period

Replication	Electrical stimulation period (minutes)				
	Control	5	10	15	20
I	42.5%	28.98%	37.23%	25.75%	46.06%
II	39.56%	42.2%	47.87%	46.23%	43.67%
III	43.63%	40.95%	29.03%	41.09%	48.14%
IV	35.8%	50%	28.57%	45.71%	29.85%
Average	40.37±3.50%	40.53±8.68%	35.68±9.05%	39.70±9.58%	41.93±8.26%

Table 4. The average of meat tenderness of muscovy duck meat stimulated electrically with different period

Replication	Electrical Stimulation Period (Minutes)				
	Control	5	10	15	20
I	2.67	2.87	2.27	2.07	1.67
II	3.27	2.6	2.2	2.27	1.53
III	3.33	3.07	2.6	1.93	1.8
IV	2.8	2.8	2.6	1.93	1.73
Total	12.07	11.34	9.67	8.2	6.73
Average	3.02±0.33$^{c1)}$	2.83±0.19^{c}	2.42±0.21^{b}	2.05±0.16^{ab}	1.68±0.11^{a}

[1)] Different superscript in the same row showed a significant difference (P<0.01)

study, the cooking loss of Muscovy duck meat ranged from 40.37 to 41.93%. (Aalhus et al. 1994), Agbeniga & Webb (2014) and Tkacz et al. (2018) which states that electrical stimulation has a negative effect on the loss of water content at the end of cooking and roasting meat products.

If compared with cooking loss of laying ducks stimulated with electricity, the cooking loss ranged from 6.99 to 21.78%. The cooking loss obtained in this study was in a normal level. This result was in accordance with Soeparno (2009) explanation that generally, the cooking loss of meat varied between 1.5 to 54.5% with a range of 15-40%.

The result showed that there were variations in the cooking loss value. Although there were variations, but these differences were not statistically significant or were considered the same. This variation could be due to the husbandry and feeding system of Muscovy duck. The ducks in this study were derived from traditional farm which their maintenance system was difficult to controlled (Hafid et al. 2015).

According to Soeparno (2009), the lower cooking loss would make the meat quality become well. This result was corroborated by Hafri et al. (2008), that meat which had lower cooking loss, under 35%, would have a good quality due to the possibility of nutrients discharge during cooking was also low.

In accordance with the statement, this research data showed that Muscovy duck meat quality was quite good if compared with laying duck meat, because the highest cooking loss in this research was 41.93%. These values belong to a good-quality level.

Tenderness

Tenderness and texture were the most important determining factor of meat quality. Consumers prefer meat that is tender because it's easier for processing and enhance the taste (Soeparno 2009). Meat tenderness is strongly influenced by the pattern of maintenance, where livestock that have physical activity such as being maintained freely without being tied up will have a larger size of muscle fibres with more and thicker connective tissue (Hafid 1998). The average of meat

Table 5. The average of muscovy duck meat's color stimulated electrically with different period

Replication	Electrical Stimulation Period (minutes)				
	Control	5	10	15	20
I	1.87	2	1.8	1.8	1.6
II	2	2	1.73	1.8	1.6
III	1.87	1.73	1.8	1.67	1.47
IV	2.2	1.87	2.4	1.4	1.47
Total	7.94	7.6	7.73	6.67	6.14
Average	1.99±0.16[b1)]	1.90±0.13[b]	1.93±0.31[b]	1.67±0.19[a]	1.54±0.08[a]

[1)] Different superscript on the same row showed a significant difference (P<0.01)

Table 6. The average score of muscovy duck meat's texture stimulated electrically with different period

Replication	Electrical Stimulation Period (minutes)				
	Control	5	10	15	20
I	3.53	2.73	2.47	1.53	1.53
II	3.6	3	2.67	1.87	1.67
III	3.6	2.93	2.2	1.8	1.47
IV	2.27	2.87	2.73	1.73	1.47
Total	13	11.53	10.07	6.93	6.14
Average	3.25±0.65[d1)]	2.88±0.11[c]	2.52±0.24[b]	1.73±0.15[a]	1.53±0.09[a]

[1)] Different superscript on the same row showed a significant difference (P<0.01)

tenderness of Muscovy duck meat stimulated with electricity were presented in Table 4.

The result of this study showed that period of electrical stimulation gave a significant effect (P<0.01) on Muscovy duck meat tenderness. Twenty minutes stimulation showed the best result on meat tenderness than 0, 5, and 10 minutes stimulation, but it was not different with 15 minutes stimulation. This result is in accordance with Davel et al. (2003) who get the influence of electrical stimulation, as well as (Yong et al. 2007) who get a shear force value of electrically stimulated meat that is lower than 10% than that which is not stimulated or 10% less. Likewise with this result was in accordance with the result that obtained (Hafid et al. 2014) who found a significant effect of 10 volt/2 minutes and 20 volt/2 minutes electrical stimulation on duck meat tenderness compared with its control.

According to Soeparno (2009), the longer the electrical stimulation also increased the value of meat tenderness. This was due to the longer the stimulation and the more glycolysis occur causing the more lactic acid formed. The formation of more lactic acid will cause a decrease in meat pH. This will cause protein denaturation and meat structure will become more tender.

Electrical stimulation can accelerate the rigormortis process through increasing glycolysis process. Glycolysis process will increase the amount of formed lactic acid. The more lactic acid, the pH of meat will decrease and cause denaturation of proteins. This will make the meat becomes more tender.

Color

Color is one factor that affect the consumer like or dislike against meat as well as the determinants of the meat quality. The meat color can be detected using sense of sight. Factors that determining meat color is concentration of meat myoglobin pigment. According to Abustam (2012) the color of meat is an important quality trait for the meat industry and household consumers. In the meat industry, the color of meat is assessed as the physical appearance of meat received by consumers and at the retail level the color of the meat causes a high level of acceptance. Consumers tend to associate color with the level of freshness of the meat. The average of Muscovy duck meat's color can be seen in Table 5.

The result showed that 15 and 20 minutes period of electrical stimulation gave a significant effect (P<0.01) on meat color. This was because of the electrical stimulation that could reduce the bonding formation of rough fibres on the muscle surface and caused the color become light. The result showed that stimulation period during 15 and 20 minutes give the best effect compared with 0, 5, and 10 minutes stimulation. Froning and Uijttenboogaart (1988) reported that the breast muscles of chicken carcasses which were electrically stimulated were significantly darker, with bright values if hot boning was done at 60 minutes or earlier.

Texture

Meat texture is a condition of meat that can be detected by mastication. The main textural characteristics of meat are firmness (toughness or

Table 7. The average score of Muscovy duck meat's flavor stimulated electrically with different period

Replication	Electrical Stimulation Period (minutes)				
	Control	5	10	15	20
I	2.93	2.53	2.4	2.2	1.67
II	2.87	2.73	2	1.93	1.8
III	2.67	2.2	2	2.13	1.67
IV	2.4	2.4	2	2.07	1.6
Total	10.87	9.86	8.4	8.33	6.74
Average	2.72±0.24 [c1)]	2.47±0.22[c]	2.10±0.20[b]	2.08±0.11[b]	1.69±0.08[a]

[1)] Different superscript on the same row showed a significant difference (P<0.01)

Table 8. The average of juiceness score of muscovy duck meat juiceness stimulated electrically with different period

Replication	Electrical stimulation period (minutes)				
	control	5	10	15	20
I	2.2	2.47	2.2	2.07	3.13
II	2.27	2.53	2	2.33	3
III	3	2.67	2.47	2.8	3.2
IV	1.73	2	1.93	3.27	3.53
Total	9.2	9.67	8.6	10.47	12.86
Average	2.30±0.52[a1)]	2.42±0.29[a]	2.15±0.24[a]	2.62±0.53[ab]	3.22±0.23[b]

[1)] Different superscript on the same row showed a significant difference (P<0.01)

degree of tenderness), cohesiveness and juiciness. The texture of meat is influenced by the cook time and temperature (Freeman & Freeman 2015; Hafid 2017). The average score of Muscovy duck meat's texture can be seen in Table 6.

The result showed that period of electrical stimulation affected significantly (P<0.01) on the texture of Muscovy ducks meat. Stimulation during 15 and 20 minutes gave the best texture if compared with 0, 5, and 10 minutes stimulation.

Muscovy duck meat that had been stimulated with electricity to different period of time had relatively different textures. In this case, panellist acceptance of Muscovy duck meat ranges from medium to very smooth textures. This is related to the level of texture roughness increased to age increasing. Muscle with small fibres does not show the texture roughness with age increasing. Muscular of male animal have a rougher textures than female animal. Types of animal also affect the muscular textures. Connective tissue of young animal contained the lower reticulin and cross-ties than the collagen of connective tissue of older animal (Soeparno 2009). Salm et al. (1981) reported that electrical stimulation significantly improved meat color, firmness and texture as well as lowering temperature in the muscles after 24 hours of postmortem.

Flavor

Flavor is one factor that determining the meat quality and can be detected by tongue. Meat flavor is a complex phenomenon related to the compounds that are soluble and volatile. Involves organ tasting and smell in its judgment. Flavors vary based on: meat cuts and the level of fat infiltration (marbling), the rate of change that occurs during maturation, some zootechnic characters and how to serve dishes (Abustam 2010; Hafid 2017). The average of flavor score of Muscovy duck meat stimulated with electricity can be seen in Table 7.

The result showed that electrical stimulation period gave a significant effect (P<0.05) on flavor of Muscovy duck meat. Twenty minutes stimulation showed the best flavor score of all treatments. This result was similar to the report of Syam et al. (2013). According to Syam et al. (2013), laying hen meat that had been stimulated using electricity with various voltage, has a relatively same flavor, so the consumer acceptance did not differ. In this case, consumer acceptance against laying hen meat ranged from rather like to like.

Prasetyo et al. (2013), Hafid et al. (2017) and Hafid (2017) explained that generally, meat flavor was affected by fat content. One of the parameters to assess the taste is to evaluate the fat content of the meat.

Juiciness

Juiciness is the ability of meat to release juices (liquid meat) during mastication. Juiciness is a factor that is considered in the assessment of meat quality, together with tenderness can explain up to more than 80% of consumers' choices in developed countries on meat quality. Soft meat in general at the first bite will produce juice that is quite significant. There is a good

correlation between the releases of meat juice with tenderness. Wetness varies based on pH, maturation and stress factors (Abustam 2010; Hafid 2017). The average of juiciness score of Muscovy duck meat juiciness are presented in Table 8.

The result showed that electrical stimulation had a significant effect ($P<0.05$) on Muscovy duck meat juiciness. Twenty minutes of electrical stimulation gave the good juiciness score and significantly different to 0, 5, and 10 minutes stimulation. However, juiciness of Muscovy duck meat stimulated during 20 minutes did not differ with those with 15 minutes stimulation. This result was similar to Abustam (2010) who explained that high voltage caused high loss of meat juice in functional properties and muscle unity compared with stimulation using low voltage. The effect of electrical stimulation might be varied depend on stimulation condition.

Meanwhile, the results of the study by Davel et al. (2003) found that the consumer acceptance score (panelists) of juiciness, tenderness, taste and overall acceptance were not significantly affected by carcass electrical stimulation. Both samples from electrically stimulated and non-stimulated carcasses are highly accepted by consumers.

CONCLUSION

Electrical stimulation significantly improves the quality of Muscovy duck meat, especially for tenderness, color, texture, aroma, taste, and juiciness of muscular duck meat, but not on pH parameters and cooking loos. Twenty minutes of electrical stimulation with a power of 20 V, showing the best effect on tenderness, color, texture, and taste.

ACKNOWLEDGEMENT

Gratitude and appreciation were given to the Dean of the Faculty of Animal Science of the University Halu Oleo, Chair of the Department of Animal Science with permission and support, as well as Animal Science laboratory technicians and students who have helped carry out the research.

REFERENCES

Aalhus JL, Jones SDM, Best DR, Robertson WM, Lutz S. 1994. The efficacy of high and low voltage electrical stimulation under different chilling regimes. Can J Anim Sci. 74:433-442.

Aberle ED, Forest JC, Hendrick HB, Judge MD, Merkel RA. 2001. Principles of meat science. San Fransisco (USA): W.H. Freeman and Company.

Abustam E. 2012. Ilmu daging: Aspek produksi, kimia, biokimia dan kualitas. Cetakan Pertama. Makassar (Indones): Masaguna Press.

Agbeniga B, Webb EC. 2014. Influence of electrical stimulation on carcass and meat quality of Kosher and conventionally slaughtered cattle. S Afr J Anim Sci. 44:58-63.

Bouton PE, Ford AL, Harris PV, Shaw FD. 1978. Effect of low voltage stimulation of beef carcasses on muscle tenderness and pH. J Fd Sci. 43:1392-1396.

Buckle KA, Edwards R, Fleet G, Wooton M. 1987. Ilmu pangan. Hari P, Adiono, translater. Yogyakarta (Indones): Universitas Indonesia Press.

Davel M, Bosman M, Webb E. 2003. Effect of electrical stimulation of carcasses from Dorper sheep with two permanent incisors on the consumer acceptance of mutton: review article. S Afr J Anim Sci. 33:206-212.

Freeman C, Freeman C. 2015. Texture analysis for assessing meat tenderness: Tenderness, juiciness, flavours. England: AMETEK Brookfield. Stadium Way, Harlow, Essex CM19 5GX.

Froning GW, Uijttenboogaart TG. 1988. Effect of post-mortem electrical stimulation on color, texture, pH, and cooking losses of hot and cold deboned chicken broiler breast meat. Poult Sci. 67:1536-1544.

Garcia L. 2009. Functional properties and utilization of high pH beef (Dissertation). [Texas (USA)]: Texas A&M University.

Gasperz V. 2010. Metode rancangan percobaan. Bandung (Indones): Penerbit CV. Armico.

Hafid H. 1998. Kinerja produksi sapi australian commercial cross yang dipelihara secara fedlot dengan kondisi bakalan dan lama penggemukan yang berbeda (Thesis). [Bogor (Indones)]: Bogor Agricultural University.

Hafid H. 2011. Pengantar evaluasi karkas. Cetakan Pertama. Kendari (Indones): Unhalu Press.

Hafid H. 2017. Pengantar pengolahan daging. Cetakan Pertama. Bandung (Indones): Alfabeta Bandung.

Hafid H, Mujianto D, Agustina, Inderawati, Nuraini. 2017. The effect of storage time in the refrigerator to the quality of organoleptic beef. ADRI Int J Biol Educ. 1:29-36.

Hafid H, Nuraini, Inderawati. 2014. Sifat organoleptik daging itik afkir yang diberi perlakuan stimulasi listrik. Prosiding Seminar Nasional Optimalisasi Sumber Daya Lokal pada Peternak Rakyat Berbasis Teknologi. Makassar (Indones): Universitas Hasanuddin. p. 182-193.

Hafid H, Nuraini, Inderawati. 2015. Potensi produksi karkas itik lokal afkir yang berasal dari peternakan rakyat di sulawesi tenggara. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 443-447.

Hafid H, Syam A. 2012. Pengaruh aging dan lokasi otot terhadap kualitas organoleptik daging sapi. Bul Peternak. 31:209.

Hafri Y, Hidayati, Elfawati. 2008. Kualitas daging sapi dengan kemasan plastik PE (polyethylen) dan plastik PP (polypropylen) Di pasar arengka kota pekanbaru. J Peternak. 5:22-27.

Khasrad, Sarbaini, Arfai, Rusdimansyah. 2018. Effect of post-mortem electrical stimulation on meat quality of Pesisir cattle (indigenous cattle of West Sumatera). Pak J Nutr. 17:441-445.

Komariah, Rahayu S, Sarjito. 2009. Physical characteristics of beef, buffalo and lamb meat on different postmortem periods. Bul Peternak. 33:183-189.

Lee S, Norman J, Gunasekaran S, van Laack RLJ, Kim B, Kauffman R. 2000. Use of electrical conductivity to predict water-holding capacity in post-rigor pork. Meat Sci. 55:385-389.

Lukman DW. 2010. Nilai pH daging. Bogor (Indones): Bagian Kesehatan Masyarakat Veteriner, Fakultas Kedokteran Hewan, Institut Pertanian Bogor.

Prasetyo H, Padaga MC, Sawitri ME. 2013. Kajian kualitas fisiko kimia daging sapi di pasar Kota Malang. J Ilmu Teknol Hasil Ternak. 8:1-8.

Randa S, Wahyuni I, Joseph G, Uhi HT, Rukmiasih, Hafid H, Parakkasi A. 2002. Efek pemberian serat tinggi dan vitamin-E terhadap produksi karkas dan non karkas itik Mandalung. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 261-264.

Salm CP, Mills EW, Reeves ES, Judge MD, Aberle ED. 1981. Effect of electrical stimulation on muscle characteristics of beef cattle fed a high energy diet for varying lengths of time. J Food Sci. 46:1284-1285.

Soekarto S, Hubeis M. 1992. Petunjuk laboratorium metode penelitian indrawi. Bogor (Indones): Pusat Antar Universitas Pangan dan Gizi, Institut Pertanian Bogor.

Soeparno. 2009. Ilmu dan teknologi daging. Yogyakarta (Indones): Gadjah Mada University Press.

Syam A, Soeparno, Rusman. 2013. Efek lama stimulasi dan tegangan listrik terhadap komposisi kimia, kualitas fisik, dan sensori daging ayam petelur afkir. Bul Peternak. 37:26.

Tkacz K, Więk A, Żywica R, Banach JK. 2018. The effects of beef carcasses high voltage electrical stimulation and roasting methods on tenderness and water retention of beef. Tech Sci. 21:103-116.

Yong SK, Lee CN, DuPonte M., Fukumoto G. 2007. Improving tenderness of forage-finished beef using a low-voltage electrical stimulator. Food Saf Technol. 22:1-6.

Author Index

Key Words Index

Jurnal Ilmu Ternak dan Veteriner

Indonesian Journal of Animal and Veterinary Sciences

ISSN: 0853-7380, E-ISSN 2252-696X Date of issue 2018-12-31

UDC: 575.113.1

Faid-Allah, E. (Department of Animal Production, Faculty of Agriculture, Menufia University, Egypt)
Ghoneim, E. (Department of Animal Production, Faculty of Agriculture, Menufia University, Egypt)
Elbetagy, A.R. (Department of Animal Biotechnology, Animal Prod. Res. Institute, Agric. Res. Center, Egypt)
El-Dabour, M. (Department of Animal Production, Faculty of Agriculture, Menufia University, Egypt)

Perbedaan dan struktur genetik populasi sapi Mesir lokal dan persilangan sapi Perancis-Mesir melalui penandaan DNA Mikrosatelit (Genetic diversity and structure of native Egyptian cattle populations and French-Egyptian Cross via DNA-Microsatellite)

(Org: Eng)

JITV 23(1): 1-10

This study investigates genetic diversity and structure of native Egyptian cattle populations, called Baladi, as Saidi from Southern Egypt, Menufi from Mid-Delta and their F1 crosses with the French Tarentaise breed using DNA Microsatellite markers. A total of unrelated 97 individuals were genotyped utilizing eight SSR primers (ETH10, ETH225, BM1818, BM1824, BM2113, SPS115, TGLA53 and TGLA126). All utilized SSR were found to be polymorphic. The highest and lowest numbers of alleles detected were 16 and 6 at TGLA53 and SPS115 loci, respectively. Baladi-Tarentaise crosses (Bal-Tar) had the highest number of alleles over all. The PIC values of 7 loci were higher than 0.5, indicating high allelic variation of utilized markers. Estimated PIC values were up to 0.898, 0.866 and 0.873 for TGLA53 genotyped in Saidi, Menufi and Bal-Tar, respectively. H_{obs} values were lower than the expected ones in the native populations accompanied with positive values for F_{is} and significant deviation from HWE indicating inbreeding trend in native populations. Structure analysis indicated three ancestral genetic backgrounds. The native populations share two main backgrounds in almost equal percentages, while the Bal-Tar had the third one. The three populations showed low percentage of admixture. The studied Mediterranean cattle populations that belong to Egypt and France seem to have differentiated from each other with only little genetic exchange between the geographically isolated populations so local cattle is very similar.

(Author)

Key Words: Cattle, Genetic Diversity, DNA Microsatellite

UDC: 616.98

Gracia, E. (Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jakarta 12930, Indonesia)
Magdalena, S. (Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jakarta 12930, Indonesia)
Wina, E. (IRIAP, Bogor)
Sinurat, A.P. (IRIAP, Bogor)
Purwadaria, T. (IRIAP, Bogor)

Aktifitas ekstraksi tanaman sebagai antioksidan dan antibiofilm terhadap bakteri saluran pencernaan ayam (Plant extract activities as antioxidant and antibiofilm against chicken gut bacteria)

(Org: Eng)

JITV 23(1): 11-17

The occurrence of microbial resistance against antibiotic due to the subtherapeutic dosage of antibiotic growth promoter (AGP) in poultry can be prevented by the antibiofilm substance. Plant secondary compounds have some activities like antioxidant, antimicrobial, and antibiofilm. This research was conducted to obtain the plant with the highest activity of antibiofilm and also antioxidant by analyzing several plant secondary compounds as antioxidant and antibiofilm against chicken's gut bacteria. The tested plants were clove leaves, leaffruit plants, mangosteen peel, cashew nut shell, guava leaves, and bay leaves. These plants were extracted with methanol or n-hexane using sonication method. The antioxidant activity as the IC50 value of the plant methanol extracts were determined using α,α-diphenyl-β-picrylhydrazyl (DPPH) assay. The biofilm inhibition activity was tested against *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus* ATCC® 29213TM using methanol and n-hexane extracts. All of the samples had antioxidant activity. The clove leaves and leaffruit plants had the highest antioxidant activity, while mangosteen peel extract in methanol had the highest antibiofilm activity against all tested bacteria. The species of bacteria also affected the antibiofilm activity. *E. coli* and *S. enteritidis* were more resistant to antibiofilm then *S. aureus*. Mangosteen peel extract which showed high antioxidant and antibiofilm activity is potential to be used as a feed additive to control the pathogenic bacteria.

(Author)

Key Words: Chicken, Plant, Secondary Compounds, Antioxidant, Antibiofilm

UDC: 591.53

Sinurat, A.P. (IRIAP, Bogor)
Wina, E. (IRIAP, Bogor)

Rakhmani, S.I.W. (IRIAP, Bogor)
Wardhani, T. (IRIAP, Bogor)
Haryati, T. (IRIAP, Bogor)
Purwadaria, T. (IRIAP, Bogor)

Zat bioaktif dari beberapa tanaman herbal dan keefektifannya sebagai antioksidan, antibakteri dan antijamur (Bioactive substances of some herbals and their effectiveness as antioxidant, antibacteria and antifungi)

(Org: Eng)

JITV 23(1): 18-27

A study was conducted to explore the bioactive substances of some local plants in order to find their effectiveness as antioxidant, antibacteria and antifungi to be used as feed additives. Twelve plants material were used in this study. The total phenol, tannin and saponin contents in the plant extract were assayed. The extracts were also assayed on their antioxidant activities and on their ability to depress in vitro gas production of microbes obtained from chicken's guts, their ability to inhibit growth of bacteria (*E. coli* and *Salmonella enteridis*) and fungi (*A. niger*). The results showed that the highest total phenol and total tannin contents were found in clove leaf extract, while the highest saponin content was found in *Sapindus rarak* fruit pericarp. The highest antioxidant activity was found in the leaffruit extract. Gas produced by microorganims was reduced to the level similar to antibiotic addition were found with addition of hexane- extract of leaffruit, kapok seed or methanol- extract of mangosteen fruit rind pulp or clove leaves. The best inhibitory effect on *E. coli* growth (measured by clearing zone) was found in methanol extract of *S. rarak* fruit. However, the most effective growth inhibitor for both E. coli and Salmonella was the liquid smoke of cashew nut shell. The best growth inhibitor for fungal growth was found in extract of clove leaves. Therefore, clove leaves extract (anti fungi), liquid smoke of cashew nut shell (antibacteria) and leaffruit (antioxidant) may have potential to produce feed additives to substitute antibiotic growth promoters

(Author)

Key Words: Bioactive Substances, Herbals, Antioxidant, Antibacteria, Antifungi

UDC: 591.53

Tesfay, G. (Department of Animal Production and Technology, Adigrat University, P.O.Box 50, Tigray, Ethiopia)
Tamir, B. (Department of Animal Production Studies, Addis Ababa University, P.O.Box 34, Bishoftu, Ethiopia)
Berhane, G. (Department of Animal Production Studies, Addis Ababa University, P.O.Box 34, Bishoftu, Ethiopia)

Substitusi tepung daun mulberry terhadap konsumsi pakan, bobot badan dan karakteristik karkas anak domba dataran tinggi Tigray (Substitution of mulberry leaf meal on feed intake, body weight and carcass characteristics of Tigray highland lambs)

(Org: Eng)

JITV 23(1): 28-37

The purpose of this study is to evaluate effects of partial or full substitution of mulberry leaf meal for concentrate mix on performances of Tigray highland lambs. Thirty intact yearlings Tigray highland male lambs (average initial body weights of 17.8±0.95 kg) were separated into 6 groups based on their live weight with each groups assigned 5 treatment diets (RCBD), that are: T1: 300 g concentrate mix alone, T2: 225 g concentrate mix + 86.55 g mulberry leaf, T3: 150 g concentrate mix + 173.1 g mulberry leaf, T4: 75 g concentrate mix + 259.7 g mulberry leaf and T5: 346.2 g mulberry leaf alone. The treatments diets were designed in such a way that concentrate mix was progressively replaced by mulberry leaf meal from 0% to 100% at iso-nitrogenous level. Lambs were adapted to experimental diets for 15 days, and after adaptation period, feeding trial was conducted. Results reveal that complete substitution of concentrate mix by mulberry leaf meal showed in higher ($P<0.05$) total dry matter, organic matter, NDF and ADF intake than the sole concentrate mix. The growth performance parameters resulted comparable across all the treatment diets. The slaughter weight and empty weight resulted higher ($P<0.05$) in sole mulberry leaf meal as compared to the whole concentrate mix supplemented lambs. On the other hand, the dressing percentage on empty body weight base and hot carcass weight showed less difference ($P>0.05$) across the different treatments. Therefore, mulberry foliage could potentially be used to replace concentrate mix as a feed supplement for the small holder farmers in Ethiopia.

(Author)

Key Words: Mulberry Leaf, Digestibily, Weight Gain

UDC: 636.597

Purba, M. (IRIAP, Bogor)
Sinurat, A.P. (IRIAP, Bogor)

Pengaruh suplementasi berbagai kadar enzim BS4 dalam ransum berbasis dedak padi terhadap performa itik pedaging PMp (Effect of supplementation of BS4-enzyme levels in rice-bran based rations on performance of growing PMp broiler duck)

(Org: Eng)

JITV 23(1) 38-44

The purpose of enzymes supplementation in feeds is to improve nutrient digestibility through degradation of anti-nutrition and crude fiber, which are commonly found in rice bran. The aim of the study was to see performance response of PMp broiler ducks to the supplementation of BS4-enzyme levels in rice-bran based rations. Two hundred and twenty four day-old ducks were allocated to 8 dietary treatments with 4 replicates, consisted of 7 ducks in each replicate. The composition of the feed treatments arranged as follows: T1 to T4 were rations with 30% of rice bran content with enzyme levels of 0, 50, 100, and 150 Unit/kg rice bran respectively. T5 to T8 were rations with 60% of rice bran content with the same enzyme levels as for T1 to T4 treatments. The ducklings were subjected to the treatments for the first four weeks. The variables observed were feed intake, weight gain and FCR. The results showed that the supplementation of BS4-enzymes

on rice-bran based rations significantly affected (P<0.05) feed intake and FCR, but not for weight gain (P>0.05). The most effective rations for feed consumption and FCR of PMp broiler duck were obtained on T4 treatment resulting in the highest body weight gain of 998 g/bird and lowest FCR of 2,64. It was concluded that the supplementation of 150 Unit/kg of BS4 enzyme in 30% rice-bran diet was the best combination level to be implemented in feeding PMP broiler ducks for the first four weeks period.

(Author)

Key Words: BS4 Enzyme, Rice Bran, PMp Broiler Duck

UDC: 616-036.22

Wardhana, A.H. (IRCVS, Bogor)
Abadi, I. (Food Security and Livestock Agency, Kediri, Indonesia)
Cameron, M.M. (Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK)
Ready, P.D. (Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK; Life Sciences Department, Natural History Museum, London, UK)
Hall, M.J.R. (Life Sciences Department, Natural History Museum, London, UK)

Epidemiologi myiasis traumatika yang disebabkan oleh lalat di Indonesia (Epidemiology of traumatic myiasis due to Chrysomya bezziana in Indonesia)

(Org: Eng)

JITV 23(1): 45-60

Epidemiology of traumatic myiasis in Indonesia was studied by the widespread collection of fly larvae from infested livestock in passive case detection surveys involving veterinary clinics. In addition, monthly data from Kediri regency in Eastern Java were analysed from 2006-2009 to explore the seasonality of myiasis. Larvae from a total of 260 cases from the nationwide survey and 341 cases from Kediri were identified. Except for 5 cases of chicken infestation due to Musca species in the nationwide survey, all other cases were exclusively caused by the Old World screwworm (OWS) fly, Chrysomya bezziana (Diptera: Calliphoridae). The monthly numbers of cases at Kediri were very variable, with cases in all months, but there was statistical evidence for an increase in cases in January and December, during the rainy season. The greatest numbers of infestations recorded were from cattle and goats. The most frequently infested sites nationwide and in Kediri were the vulva and umbilicus, associated with calving, which is a major risk period for traumatic myiasis. Mitochondrial DNA typing of 176 specimens was useful for detecting multiple infestations, but no association was found between genetic lineage and host. The equatorial climate of Indonesia, combined with poor husbandry systems are factors that help to support OWS fly development year round. Even if not considered a disease of strategic importance, screwworm myiasis remains a threat to livestock production in Indonesia and a major welfare issue that requires constant interventions by farmers. The new and collated epidemiological data presented represent the most extensive survey of traumatic myiasis in Indonesia to date and provide a valuable baseline to support integrated pest management programmes.

(Author)

Key Words: Myiasis, Mitochondria, *Chrysomya bezziana*, Epidemiology

UDC: 581.199

Saenab, A. (IRIAP, Bogor)
Wiryawan, K.G. (Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University)
Retnani, Y. (Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University)
Wina, E. (IRIAP, Bogor)

Manipulasi fermentasi rumen oleh produk bioindustri cangkang biji mete (*Anacardium occidentale*) untuk menekan produksi metana (Manipulation of rumen fermentation by bioindustrial products of cashew nut shell (*Anacardium occidentale*) to reduce methane production)

(Org: Eng)

JITV 23(2): 61-70

One of the strategies to reduce methane produced by ruminants is by the application of feed additive from plant materials. One of the potential plants is cashew plant especially its shell. The cashew nut shell can be processed to become 3 bioindustrial products; ie biofat, biochar, biosmoke. The aim of this research was to evaluate the effectively of biofat, biochar and biosmoke in reducing methane and other end product of rumen fermentation. The experiment was arranged in block randomize design based on time series of in vitro to evaluate 3 levels and 3 types of bioindustrial. The treatments were Control (substrate=S), Biofat: S+0.25 µL/mL, S+0.5 µL/mL, S+0.75 µL/mL; Biochar: S+0.1 mg/mL, S+0.2 mg/mL, S+0.3 mg/mL; Biosmoke: S + 2.5 µL/mL, S+5.0 µL/mL, S + 7.5 µL/mL. Each treatment was done in duplicates and the in vitro experiment was repeated 4 times. The research measured total gas production; methane production; digestibility of dry matter, organic matter and neutral detergent fibre (NDF); ammonia concentration (NH3); partial and total volatile fatty acids (VFA) concentration. The results showed that biofat, biochar and biosmoke reduced methane production by 43.88%, 24.21%, 37.88% at the highest level of inclusion, respectively. The NH3 concentration slightly increased by biochar and biosmoke addition. Molar proportion of acetic acid decreased, while molar proportion of propionic acid increased by addition of the three bioindustrial products. Organic matter digestibility decreased significantly with biofat and biosmoke addition. The mechanism of biofat, biochar and biosmoke in affecting rumen fermentation was different. It can be concluded that the three bioindustrial products of cashew nut shell can be utilized as feed additive to reduce methane and increase propionic acid production in the rumen.

(Author)

Key Words: Cashew Nut Shell, Biofat, Biochar, Biosmoke, Rumen Fermentation, Methane

UDC: 591.53

Wina, E. (IRIAP, Bogor)
Widiawati, Y. (IRIAP, Bogor)
Tangendjaja, B. (IRIAP, Bogor)

Performans sapi perah anak yang diberi pakan mengandung kalsium-asam lemak sawit dan buah *Sapindus rarak* (Performance of dairy calves fed diet containing Ca-palm oil fatty acid and *Sapindus rarak* fruit)

(Org: Eng)

JITV 23(2): 71-76

Calcium salts of palm oil fatty acid (Ca-FA) is a high dense energy source that is protected from degradation in the rumen. *Sapindus rarak* fruits (SrF) contain high level of saponin and have been reported to increase body weight sheep or cattle and reduced protozoa population in the rumen. This experiment used a combination of Ca-FA and SrF to improve the performance of weaned dairy calves. Thirty two heads of calves of Indonesian Holstein were used in factorial block design experiment (2 x 2). The first factor was Ca-FA (with 2.5% and without addition of Ca-FA) while the second factor was SrF (with 0.3% and without addition of SrF in total diet) and sex of the animal as block was applied. The feed as total mixed ration contained crude protein (CP) 17%, total digestible nutrien (TDN) minimum 69%, Net energy for maintenance 1.70 Mcal/kg and Net energy for gain 1.03 Mcal/kg. It was fed 3% of body weight of the animals for 14 weeks included 2 weeks of adaptation period. Feces collection for measuring digestibility of feed was conducted at the end of experiment. Average daily gain (ADG) of calves received SrF was higher than without SrF (896.9 vs 853.7 g/day) while ADG received CaFA was lower than without CaFA (860.6 vs 890 g/day) but both effects were not significantly different (P>0.05). DM intake due to SrF treatment tended to be higher than control treatment (4.4 vs 4.3 kg/day). DM digestibility was not different among treatments. In conclusion, calves received supplementation of 0.3% SrF fruit in the diet had similar average daily gain with those given unsupplemented diet but had reduced diarrhea cases. The use of Ca palm oil fatty acid as a dense energy source at 2.5% in the diet did not show any negative effect on calves performance.

(Author)

Key Words: Calcium-Palm Oil Fatty Acid, *Sapindus rarak*, Saponin, Dairy Calves

UDC: 591.53

Khanifah (Faculty of Animal and Agricultural Science of Diponegoro University, Semarang)
Suthama, N. (Faculty of Animal and Agricultural Science of Diponegoro University, Semarang)
Wahyuni, H.I. (Faculty of Animal and Agricultural Science of Diponegoro University, Semarang)

Pemanfaatan protein ransum pada ayam broiler akibat penambahan glukonaman dari ekstrak umbi porang (*Amporphopallus onchophyllus*) (The effect of glucomannan inclusion derived from porang tuber extract (*Amorphophallus oncophyllus)* on dietary protein utilization in broiler chicken)

(Org: Eng)

JITV 23(2): 77-81

The aim of this study was to evaluate the effect of glucomannan inclusion derived from porang (Amorphophallus oncophyllus) tuber extract (GPTE) on production performance of broiler chicken. A total of 160 one day old of New Lohmann broiler chickens with an average initial body weight of 42.08±0.86g were used in this study. The study was arranged in a completely randomized design with 5 treatments and 4 replications (8 birds each). The treatments applied were T0 = basal ration, T1 = T0 + 0.05% GPTE, T2 = T0 + 0.10% GPTE, T3 = T0 + 0.15% GPTE and T4 = T0 + 0.20% GPTE. The parameters observed were protein digestibility, nitrogen retention, meat calcium and protein mass. The results showed that dietary addition of GPTE significantly (P<0.05) increased the coefficient of protein digestibility, meat calcium and protein mass, but had no effect on nitrogen retention. The highest protein digestibility and meat calcium mass were shown in T4, but they were not significantly different from those in T3, and T2 for protein digestibility. While the highest meat protein mass was in T2 but it was not different with than in T3 and T4. The conclusion of the study is that dietary supplementation of glucomannan derived from porang tuber extract in broiler up to 0.15% (T3) increases protein digestibility, meat calcium and protein mass, and daily weight gain with similar nitrogen retention.

(Author)

Key Words: Glucomannan, Protein Digestibility, Nitrogen Retention, Calcium Mass, Protein Meat, Broiler

UDC: 637.112

Yanthi, N.D. (Postgraduate student of Production Science and Livestock Technology, Bogor Agricultural University; Biotechnology Research Center, IIS, Cibinong, Bogor)
Said, S. (Biotechnology Research Center, IIS, Cibinong, Bogor)
Anggraeni, A. (IRIAP, Bogor)
Damayanti, R. (Faculty of Animal Health, Bogor Agricultural University)
Muladno (Faculty of Animal Husbandry, Bogor Agricultural University)

Korelasi nilai electric conductivity dengan kualitas susu sapi perah (Correlation of electric conductivity values with the dairy milk quality)

(Org: Eng)

JITV 23(2): 82-88

Milk, as the prime source of food for mammals, has an electrolyte to replace the loss of body fluid caused by activity or metabolism process. The total electrolyte concentration can be measured based on conductivity value from the

nutritional content. Therefore, the parameter of the quality of milk with conductivity value can be a benchmark for quality and selling value of milk, making it simpler to be implemented in the field. The aim of this research is to analyze the relation between electric conductivity (EC) with the content value of cow milk. The milk was taken from 10-30 cows from a farm in Lembang (district of West Bandung), Pengalengan (district of Bandung), Tasikmalaya, Sumedang, Subang, Sukabumi and Bogor of West Java Province. The milk was put in 50 ml of sterile falcon. The Probe EC count-meter CT-3031 was used to measure EC while the quality of milk was measured by Probe MilkoScanTMFT 120 (Foss). The milk quality is reflected by protein content, Fat, Total Solid (TS), Solid Non-fat (SNF), Lactose, Density, Acidity and Freeze Point Deviation (FPD). The results of this study show that the EC value in the milk gives a very real positive effect ($P \leq 0.01$) to Total Solid (TS), Solid Non-fat (SNF), Lactose, and Freeze Point Deviation (FPD). The value of EC also significantly affect ($P \leq 0.05$) the value of density in milk. Therefore, the value of EC can be used to predict the quality value of milk.

(Author)

Key Words: Correlation, Electric Conductivity, Milk Quality

UDC: 665.637

Widiastuti, R. (IRCVS, Bogor)
Anastasia, Y. (IRCVS, Bogor)

Residu clenbuterol pada daging sapi yang dikoleksi dari beberapa kota di Pulau Jawa, Indonesia (Clenbuterol residue in beef meat collected from several cities In Java Island, Indonesia)

(Org: Eng)

JITV 23(2): 89-94

Clenbuterol (CLB) is an illegally animal drug of the β-agonist group that used as a promoter of growth in various farm animals. The presence of CLB residues in livestock products can cause poisoning in humans, such as tremor, tachycardia, nausea and dizziness. The purpose of this research is to validate CLB residue detection method on beef meat detected using a high performance liquid chromatography (HPLC) and to determine the presence of CLB residue on 74 samples of frozen and fresh beef meat from several cities in Java. Samples were extracted with acetonitrile and isopropanol, then analyzed chromatographically using RP ODS C18 column and mixed mobile phases of 50 mM NaH2PO4 and acetonitrile (80:20, v/v) and detected by photodiode array detector at a wavelength of 210 nm. The recoveries were 103.45, 89.27 and 89.53% for each additional spiked at concentrations of 2, 5 and 10 ng/g of CLB. The detection limit and the quantitation limit were 0.10 ng/g and 0.31 ng/g, respectively. Analysis of 74 samples showed that CLB residue was detected in 8 samples in a concentration range of 2.40 to 15.06 ng/g and had exceeded the CLB residue maximum limit of 0.2 ng/g. To avoid the risk of the presence of CLB residues, it is necessary to regularly monitor the presence of residue of animal products to ensu re of food safety for consumers.

(Author)

Key Words: Clenbuterol, Residue, Beef Meat, HPLC

UDC: 633.37

Manpaki, S.J. (Graduate School of Nutrition and Feed Science, Bogor Agricultural University, Bogor 16680, Indonesia)
Prihantoro, I. (Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor 16680, Indonesia)
Karti, P.D.M.H. (Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor 16680, Indonesia)

Respon pertumbuhan kalus embriogenik tanaman lamtoro terhadap perbedaan umur embrio dan hormon Auksin 2,4-Dichlorophenoxyacetic acid (Growth response of leucaena embryogenic callus on embryo age differences and Auxin 2,4-Dichlorophenoxyacetic acid)

(Org: Eng)

JITV 23(2): 95-102

Leucaena (*Leucaena leucocephala* cv. Tarramba) is a source of protein from the legume family. Tarramba varieties able to adapt well in tropical area such as West Nusa Tenggara and East Nusa Tenggara. This study aimed to identify the growth response and embryogenic callus morphology of lamtoro (*L. leucaena* cv. Tarramba) in embryos different ages and auxin 2,4-D levels. This research was used explants derived from lamtoro (*L. leucocephala* cv. Tarramba) as much 400 explants. this study conducted of 16 treatments with two factors, the first factor is the provision of PGR 2,4-D concentration of 0.5 mg/L, 1 mg/L, 1.5 mg/L and 2 mg/L. The second factor was the type of embryo such as mature embryo, cotyledon, heart, and pre-globular. Each treatment was repeated 25 replications. This study uses a randomized complete design (CRD) with two factors. Data were analyzed using analysis of variance and if there was significant difference, data were further analyzed using Duncan's multiple range test. Analysis of variance showed that PGR 2,4-D at a concentration of 1.5 mg/L and the type of optimum embryo was mature embryo callus on parameters as height, diameter increment callus, and callus color scores were significantly different ($P<0.05$). callus texture parameter indicates results that are uniform throughout the treatment that was compact callus. Lamtoro plant embryogenic callus (*L. leucocephala* cv. Tarramba) indicate an optimal response at the concentration of PGR 2,4-D 1.5 mg/L and the type of embryo was mature embryo.

(Author)

Key Words: Auxin 2,4-D, Embryo, Embryogenic, Callus, Lamtoro cv. Tarramba

UDC: 577.175.2/.7

Mourad, R.S. (Animal Production Department, Faculty of Agriculture, Menufiya University, Egypt)

Komponen biokimia darah dan hormon progesteron pada estrus sapi persilangan di Mesir (Blood biochemical components and progesterone hormone on day of estrus in crossbred cattle in Egypt)

(org: Eng)

JITV 23(3): 103-111

Deficiencies or excess minerals such as P, Cu, and Zn are associated with subnormal fertility and anoestrus conditions in cows. This study was conducted in a veterinary unit in Menufiya, Egypt. Eighteen head of crossbred cows were randomly selected at random at estrus time (estrus group) and as a control 14 head of crossbred cows were selected from newly-calving dams at about 6-12 hours after calving (control group). The aim of this study was to use the components of biochemistry and progesterone hormone on estrus day of crossbreeding cattle in Egypt. The information from this study will be used to confirm estrus time to improve mating percentage. In blood plasma, the concentrations of all biochemical components and progesterone concentrations in estrus animals are higher than control cows except globulin. The results showed that blood plasma from control animal obtained Mg, Ca / P ratio, Co, Cu, Zn, Se, and Mo concentration is higher than estrus cattle. On the other hand, blood plasma concentrations of Na, K, Ca, P, Mn, and Fe are higher in estrus cattle. The progesterone concentration of estrus cattle is lower during summer than in winter. In estrus cows, higher plasma macro-elements were found in winter than in summer except for K, Ca and Ca/P ratio. On the other side of estrus cattle, all trace elements of blood plasma are higher in winter than in summer except Zn, Mn, Se, and Fe.

(Author)

Key Words: Cattle, Glucose, Cholesterol, Minerals, Progesterone

UDC: 616-008.87

Pasaribu, T. (IRIAP, Bogor)
Sinurat, A.P. (IRIAP, Bogor)
Wina, E. (IRIAP, Bogor)
Purwadaria, T. (IRIAP, Bogor)
Haryati, T. (IRIAP, Bogor)
Susana, I.W.R. (IRIAP, Bogor)

Efektifitas campuran bahan bioaktif beberapa tanaman dalam menghambat pertumbuhan bakteri *Escherichia coli* dan *Salmonella sp* (Effectiveness of bioactive combinations of several plant substances to inhibit the growth of *Escherichia coli* and *Salmonella* sp.)

(Org: Eng)

JITV 23(3): 112-122

The use of antibiotic growth promoters (AGP) has been banned as feed additives in many countries, therefore the alternatives need to be found. An in vitro experiment was conducted to study the potential of combination of some plant extract to inhibit growth of pathogen bateria that normally occur in the poultry gastro intestinal tract and in vivo studies to evaluate the population of *E. coli* in the ileum, the immune response and blood profile of chicken. The combination of three plants bioactives (liquid smoke of cashew shells of Anacardium occidentaleor CLS, Phyllanthus niruri L. extract (EM), and Synzygium aromaticum extract (EDC) were formulated and evaluated for its effectiveness to inhibit growth of *Escherichia coli* and *Salmonella* sp. *in vitro*.The mixtures (KE) were then made in 3 different concentrations, i.e. 100 % KE, 50% KE, and 25% KE and studied their effectiveness to inhibit growth of E. coli or Salmonella sp. using microplate reader method. In biological assay, the bioactive combination was at a concentration of 0.0625% CAM + 0.0625% EM + 0.0313% EDC. The treatment consisted of 8 types of rations, each of it 2 replications and each replication consisted of 5 DOC. At the end of the experiment (35 days), blood was taken from 2 chickens at each replication. The results showed that the higher the concentration of the bioactive combination (KE100) the higher the ability to inhibit the growth of *E. coli* or *Salmonella* sp. The combination of bioactive substances CAM, EM, and EDC more effectively than Zn-bacitracin antibiotics to inhibit the growth of *E. coli* and *Salmonella* sp. The optimum concentration of KE with the similar effectiveness as the AGP was 25%. It was concluded that the combination of CAM, EM, and EDC was able to inhibit the growth of *E. coli* and even capable to eliminate the presence of *Salmonella* sp. In the biological assay, a combination of CAM, EM, and EDC either extract or powder form, high dose, medium or low does not affect the weight of the spleen, bursa fabricius, and blood profile. The best combination of CAM, EM, and EDC extracts to decreases the total bacterial population and E. coli was middle dose ie 0.0625% (extract) and 0.625% (powder). Likewise for live weight gain that was a good dose of extract or powder form can replace antibiotics. It was concluded that combinations of CAM, EM, and EDC had potential as a substitute for AGP in poultry feed, especially chickens.

(Author)

Key Words: *Anacardium occidentale, Phylanthus niruri L., Synzygium aromaticum, E. coli, Salmonella sp*

UDC: 616.15

Gustian (Faculty of Veterinary Medicine, Bogor Agricultural University)
Soehartono, H. (Faculty of Veterinary Medicine, Bogor Agricultural University)
Jujur, N. (Center for Technology Services, Agency for the Assessment and Application of Technology)
Wargadipura, A.H.S. (Center for Technology Services, Agency for the Assessment and Application of Technology)
Noviana, D. (Faculty of Veterinary Medicine, Bogor Agricultural University)

Profil darah implantasi stainless steel 316L bahan implan lokal pada tulang femoralis tikus (Blood profile of implantation stainless steel 316L local implant material on rat femoral bone)

(Org: Eng)

JITV 23(3): 123-129

This study was aimed to obtain information regarding complete blood count (CBC) profile of post implantation of

stainless steel (SS) 316L as an Indonesian local product of non-degradable metal implant. Thirty adult male rat, aged approximately 12 weeks were divided into 3 groups, i.e. control group without implantation, implant group with import SS316L and implant group with Indonesian national local SS316L that developed by Agency for the Assessment and Application of Technology (BPPT). The implant groups were given implants by inserting it between femoral bone and biceps femoris muscle. On the control group, defect was made on bone without inserting an implants material. Examination of the systemic response was done with CBC before and 30 days after implantation. The analysis of red blood cells amount, haemoglobin level, haematocrite value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell and its differentiation from each group did not show significant differences. In conclusion, stainless steel 316L of import and national local product showed non-negative effects on blood profile.

(Author)

Key Words: Stainless Steel, Metal Implant, Biocompatibility, Blood Profile, Foreign Body Reaction

UDC: 599.742.7

Pertiwi, A.P. (Profesional Education Program for Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Agatis Kampus IPB Dramaga, Bogor, Jawa Barat 16680, Indonesia)
Tumbelaka, L.I.T.A. (Department of Clinic Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Agatis Kampus IPB Dramaga, Bogor, Jawa Barat 16680, Indonesia)
Ulum, M.F. (Department of Clinic Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Agatis Kampus IPB Dramaga, Bogor, Jawa Barat 16680, Indonesia)

Ultrasonografi dan diagnosa sitologi vagina dari kucing betina (Ultrasonographic and vaginal cytological diagnostics of the Queen)

(Org: Eng)

JITV 23(3): 130-142

Ultrasonography is a diagnostic method to image the conditions of reproductive organs and it could be supported by vaginal cytology to identify the activities of the ovaries by the types of vaginal exfoliate cells. The aims of this study was to observe reproduction organ through ultrasonography with supportive diagnostic with vaginal cytological assessment. A total of 10 individual queens were used in this study and then grouped into intact group (n=5) and spayed (ovariohysterectomy) group (n=5) based on the anamneses or their medical history. The vagina, cervix, uterus body and horns, and ovaries were imaged and measured by ultrasound. Vagina, uterine body and horn seem as pipe-like structures with hyperechoic outer lines. The lumen in uterine body and horn seem as a hyperechoic structure. The ovaries seem as round- or oval-shaped structures with anechoic follicles. The corpus luteal has thick wall and seen as anechoic in its centre part. The corpus albicans seems as a hyperechoic structure. The vagina of spayed queens seemed more corrugated than those intact queens. The cervix is seen as a hyperechoic structure linking the vagina and uterine body. Exfoliate vaginal epithelial cell types were then also be identified and counted on each queens. The results of vaginal cytology showed that proestrus occured in 3 intact queens, late metestrus in 1 intact and 3 spayed queens, anestrus in 1 spayed queen, and unidentifiable estrus stage in 1 intact and 1 spayed queens. Moreover, the morphology of cervix and uterine was affected by the activity of ovary.

(Author)

Key Words: Sorghum, Cultivar, Biomass Production, Quality

UDC: 613.287.5

Widiastuti, R. (IRCVS, Bogor)
Anastasia, Y. (IRCVS, Bogor)

Residu aflatoksin M1 pada susu sapi segar dari peternakan sapi rakyat di Indonesia (Aflatoxin M1 residue in fresh dairy milk from small individual farms in Indonesia)

(Org: Eng)

JITV 23(3): 143-149

This present study was aimed to investigate the presence of aflatoxin M1 (AFM1) residue in fresh dairy milk collected from small dairyl farms. A total of 104 samples of fresh cow's milk were collected in Pengalengan-Bandung and Sukabumi (West Java province), and Tanggamus (Lampung province) in April and September 2012. All samples were analyzed by a high performance liquid chromatography and detected with fluorescence detector after extraction with organic solvents. Contamination of AFM1 was found on 1.96% (1/51) from the samples collected in April 2012 at concentration of 1.20 ng/L and 39.63% (21/53) from the samples collected in September 2012 at concentration of 1.20 ng/L 1.0 – 34.1 ng/L. Those positive samples were obtained from Pangalengan and Sukabumi, but none for those samples collected from Tanggamus both on collection time April nor September 2012. In those positive samples for AFM1, there is no sample contained AFM1 above the maximum level (ML) regulated in Indonesia (500 ng/L or 0.5 µg/L). Low contamination levels of AFB1 in the range of 0.38 to 6.64 µg/kg found in supplemental feed samples from the same sampling time and locations. The findings of AFM1 contamination in raw fresh milk from this study caused no harm to the consumers. However, regular monitoring on the presence of AFM1 in dairy milk and aflatoxin B1 (AFB1) in dairy cattle feed is necessary to ensure the protection of human health.

(Author)

Key Words: Residue, Aflatoxin M1, Milk, Small Individual Farms, HPLC

UDC: 615.076

Melia, S (Faculty of Animal Science, Andalas University, Padang)

Yuherman (Faculty of Animal Science, Andalas University, Padang)
Ferawati (Faculty of Animal Science, Andalas University, Padang)
Jaswandi (Faculty of Animal Science, Andalas University, Padang)
Purwanto, H. (Faculty of Animal Science, Andalas University, Padang)
Purwati, E. (Faculty of Animal Science, Andalas University, Padang)

Kualitas nutrisi dan kandungan mikrobiologi pada susu kerbau, sapi dan kambing dari Sumatera Barat (Nutrition quality and microbial content of buffalo, cow, and goat milk from West Sumatera)

(Org: Eng)

JITV 23(3): 150-157

The aim of this research was to determine the quality of fresh milk physically, chemically and microbiologically obtained from cow, goats and buffalo in West Sumatra. The research method applied was laboratory experimental to analyze nutritional value, the number of aerobic bacteria and lactic acid bacteria, isolating and identifying lactic acid bacteria. Results showed that the nutritional value of milk had meet the requirements of Indonesian National Standardization, but the total colony of aerobic bacteria was above the allowed threshold of 1 x 106 CFU/ml. In addition, each sample had a total colony of varied lactic acid bacteria (LAB). The lowest total LAB value obtained in cow's milk was 0.84±0.18 x107 CFU/ml, in contrast to buffalo milk and goat milk which had a higher total LAB of 36.8±17.57 x107 CFU/ ml and 57.25±8.89 x107 CFU/ml. However, all the colonies showed almost identical morphology of LAB isolates. It is concluded that fresh milk from West Sumatra contains LAB therefore sanitation control is still needed during handling of milk.

(Author)

Key Words: Fresh Milk, Nutrients, Total Plate Count, Lactic Acid Bacteria

UDC: 636.2.034

Faid-Allah, E. (Department of Animal Production, Faculty of Agriculture, Minuofiya University, Egypt)

Estimasi nilai breeding produksi susu dan sifat mastitis sapi holstein di Mesir (Estimating breeding values for milk production and mastitis traits for Holstein cattle in Egypt)

(Org: Eng)

JITV 23(4): 159-167

This study was carried out to evaluate the sires and dams genetically for milk production and mastitis traits in Egyptian 12 herds of Holstein cattle using Best Linear Unbiased Prediction via MTDFREML program. The data was obtained from a commercial farm called Dena, located in Cairo-Alex Desert Road (80 Km), Menofia, Egypt. Data included 4791 cows, 4227 dams and 248 sires that represented the period from 2007 to 2014. Estimating breeding values for milk production traits as cumulative milk yield at 90 days (90-DM), cumulative milk yield at 180 days (180-DM), cumulative milk yield at 270 days (270-DM), cumulative milk yield at 305 days (305-DM), and number of mastitis infection around the season of lactation (MAST). The averages of the 90-DM, 180-DM, 270-DM, 305-DM and MAST were 3026.3±655.1 kg, 5873.3±1081.1 kg, 7891.1±2692.2 kg, 9611.2±1897.9 kg, and 0.712±1.2 time/parity, respectively. Estimates of heritability for the previous traits were 0.11±0.016, 0.15±0.014, 0.18±0.012, 0.22±0.015, and 0.09±0.029, respectively; genetic variance were 47206.2 kg, 175300.6 kg, 1304654.4 kg, 792411.6 kg and 0.12 time/parity, respectively; and phenotypic variance were 429147.6 kg, 1168670.6 kg, 7248079.9 kg, 3601870.9 kg, and 1.35 time/parity, respectively. The EBV values as average, SD, (Min: Max) for sires were 0.0±0.179 (-0.4: 0.66) for MAST, 0.0±86.176 (-263.1: 245.4) for 90-DM, 0.0±227.523 (-600.3: 800.3) for 180-DM, 0.0±413.48 (-323.3: 1277.7) for 270-DM and 0.0±440.26 (-1280.9: 1565.1) for 305-DM. Also, The EBVs for dams were 0.0±0.055 (-0.14: 0.45) for MAST, 0.033±26.24 (-142.8: 103.0) for 90-DM, 0.074±76.81 (-360.2: 289.6) for 180-DM, -0.045±139.66 (-591.9: 529.2) for 270-DM and 0.266±154.1 (-666.3: 617.6) for 305-DM. These results provide that the selection of sires and dams will improve the traits of milk production and mastitis in this herd because of the wide differences in genetic potential among sires and dams.

(Author)

Key Words: Heritability, Breeding Value, BLUP, Milk Production, Mastitis, Holstein Cattle

UDC: 636.082

Margawati, E.T. (Research Center for Biotechnology, Indonesian Institute of Science, Jalan Raya Bogor Km.46, Cibinong, Bogor 16911)
Volkandari, S.D. (Research Center for Biotechnology, Indonesian Institute of Science, Jalan Raya Bogor Km.46, Cibinong, Bogor 16911)
Indriawati (Research Center for Biotechnology, Indonesian Institute of Science, Jalan Raya Bogor Km.46, Cibinong, Bogor 16911)
Ridwan, M. (Research Center for Biotechnology, Indonesian Institute of Science, Jalan Raya Bogor Km.46, Cibinong, Bogor 16911)

Keragaman genetik dan hubungan genetik diantara sapi Bali dari beberapa lokasi di Indonesia berdasarkan Marker Mikrosatelit ETH10 (Genetic diversity and relationship among Bali cattle from several locations in Indonesia based on ETH10 Microsatellite Marker)

(Org: Eng)

JITV 23(4):168-173

Bali cattle is one of local beef cattle in Indonesia, up to present its performance indicated an inbreeding occurrence. This study was aimed to analyze the genetic diversity and relationship among Bali cattle from several locations in Indonesia based on ETH10 microsatellite marker. Ninety-four (94) DNA samples (89 Bali cattle; 5 Banteng) were analyzed. The Bali cattle samples were from 6 locations in Indonesia

(15 Pulukan; 15 Nusa Penida; 14 Bima West Nusa Tenggara/WNT; 10 Mataram, WNT; 20 Riau; 15 South Borneo). DNA Banteng samples were collected from Prigen Malang of East Java. Microsatellite marker of ETH10 labelled HEX was used for amplification. Alleles were analyzed by using Cervus 3.0.7 and GenAlex 6.5. Result showed that there were five (5) alleles found in ETH10 marker *i.e.,* 209; 213; 215; 217; and 219 bp. Average of observed (Ho) and expected (He) heterozygosity were 0.46±0.05 and 0.60±0.03, respectively. Five (5) out of 6 locations were in breeding occurrence except Bali cattle from Mataram was not inbreeding. The longest genetic relationship was between Bali cattle from Mataram and Riau whereas the closest distance was Bali cattle from South Borneo and Mataram. Banteng was closest to Bali cattle from Nusa Penida and the longest was to Bali cattle from South Borneo. This finding indicates there is inbreeding in Bali cattle, therefore it needs to be concerned in bull rotation and semen distribution for increasing the Bali cattle performance.

(Author)

Key Words: Bali Cattle, ETH10 Microsatellite, Genetic Diversity, Genetic Relationship

UDC: 636.2.033

Putra, W.P.B. (Research Center for Biotechnology - Indonesian Institute of Sciences, Indonesia)
Nugraheni, S.T. (Department of Biology, Faculty of Life Sciences - Jakarta State University, Indonesia)
Irnidayanti, Y. (Department of Biology, Faculty of Life Sciences - Jakarta State University, Indonesia)
Said, S. (Research Center for Biotechnology - Indonesian Institute of Sciences, Indonesia)

Genotyping pada gen Insulin-like Growth Factor 1 (IGF1/SnaBI) sapi Pasundan dengan metode PCR-RFLP (Genotyping in the Insulin-like Growth Factor 1 (IGF1/SnaBI) gene of Pasundan cattle with PCR-RFLP method)

(Org: Eng)

JITV 23(4): 174-179

The Insulin-like Growth Factor 1 (IGF1) gene is important to control sceleton and muscle development. Therefore, IGF1 gene was widely used as the candidate gene for livestock selection. This research was carried out to identify the genotype of IGF1 gene (exon 1) using PCR-RFLP method with SnaBI restriction enzyme (TAC*GTA). Total of 90 DNA samples of Pasundan cows from Ciamis and Pangandaran Regencies were used in the present study. Research reveals that all sample in the animal studied have CC genotype with C allele as the common allele in IGF1/SnaBI gene. The CC genotype that obtained in the present study was conducted by the transition mutation position g.218T/C (GenBank: KF202095). This mutation was changed the amino acid from methionine (AUG) to valine (GUG). It was concluded that IGF1/SnaB1 gene of Pasundan cattle is monomorphic and can not used for molecular selection.

(Author)

Key Words: IGF1/SnaB1 Gene, Pasundan Cattle, PCR-RFLP, Monomorphic, Mutation

UDC: 636.39

Affan, A.A. (Department of Veterinary Preclinical Sciences)
Amirul F.M.A. (Department of Veterinary Preclinical Sciences)
Ghani, A.A.A. (Department of Veterinary Preclinical Sciences)
Annas, S. (Research Centre for Ruminant Disease; Department of Veterinary Laboratory Diagnostic, Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor)
Zamri-Saad, M. (Research Centre for Ruminant Disease; Department of Veterinary Laboratory Diagnostic, Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor)
Hassim, H.A. (Research Centre for Ruminant Disease; Department of Veterinary Preclinical Sciences)

Profil biokimiawi serum, asam hormon dan asam lemak selama akhir kebuntingan dari kebuntingan ketosis pada kambing Boer persilangan (Serum biochemical, hormonal and fatty acid profiles during the late gestation of pregnancy ketosis in Boer cross goats)

(Org: Eng)

JITV 23(4): 180-188

Pregnancy ketosis has been recognized as one of the common metabolic disease affecting goat's meat and milk production. For the present study, sixteen (n=16) individuals of pregnant does at day 80 of pregnancy had been used. A total of 8 does were categorized as control group (healthy pregnant goats), were fed on Napier grass and goat concentrate with water ad libitum, and another 8 does were considered as treatment group which categorized as ketosis based on the clinical signs and presence of ketone body in urine. Blood sample were collected from all goats for biochemical profiles analysis which were glucose, Beta-hydroxybutyrate (BHBA), free fatty acid (FFA), calcium, electrolytes (sodium, potassium, chloride), liver enzyme and hormonal levels (cortisol and insulin). Three does from each group were slaughtered and liver samples were collected for fatty acid profiles study. In this study, the BHBA, FFA, calcium, amino aspartate transferase (AST), gamma glutamyl transferase (GGT) and cortisol hormone were significantly higher in pregnancy ketosis goats as compared to control group. Meanwhile, the concentration of glucose, sodium, potassium, chloride and insulin hormones were lower in pregnancy ketosis goats as compared to control. Furthermore, the fatty acid composition in blood plasma of pregnant goat with ketosis showed higher level of palmitic, stearic and oleic acid, while in liver, palmitic, oleic and linoleic acid was found higher.

(Author)

Key Words: Beta-Hydroxybutyrate, Blood Sample, Fatty Acid, Glucose, Liver

UDC: 616.98

Widaningrum (ICAPOSTRD, Bogor)
Miskiyah (ICAPOSTRD, Bogor)
Indrasti, D. (Department of Food Technology, Bogor Agricultural University)
Hidaya, H.C. (Department of Food Technology, Bogor Agricultural University)

Peningkatan viabilitas bakteri *Lactobacillus casei* dan *Bifidobacterium longum* dengan beberapa materi enkapsulasi menggunakan metode ekstruksi (Improvement of viability of *Lactobacillus casei* and *Bifidobacterium longum* with several encapsulating materials using extrusion method)

(Org: Eng)

JITV 23(4): 189-201

Lactobacillus casei and *Bifidobacterium longum* are probiotics commonly applied as dry starter for food system. Drying process in the production of dry starter can reduce the number of probiotics, therefore they are necessary to be encapsulated. Aim of this research was to obtain best encapsulating material for both probiotics. Encapsulation technique used in this research was extrusion with maltodextrine-alginate, sago starch-alginate, corn starch-alginate, and control of treatment skim milk-alginate (w:w) as encapsulating materials. The four encapsulating materials significantly affected the value of viability, encapsulation efficiency, number of cell in wet beads and dry beads, number of survival cell during drying process, and number of survival encapsulated cell in simulated acid and bile salt conditions. Based on viability of *L. casei* and *B. longum*, beads matrix characteristic, number of cell in wet beads and dry beads, and number of survival cells during drying process, maltodextrine-alginate was better than sago starch-alginate and corn starch-alginate, but was not as good as skim milk-alginate (control of treatment) as encapsulating material. Viability (number) of *L. casei* and *B. longum* in dry beads of maltodextrine-alginate were 4.69±0.08 log CFU/g and 5.32±0.21 log CFU/g, while number of *L. casei* and *B. longum* in dry beads of skim milk-alginate were higher 5.08±0.07 log CFU/g and 6.20±0.16 log CFU/g. *L. casei* more resistant than *B. longum* against acidic (low pH) environment. In the presence of 0.3% bile salt, *L. casei* and *B. longum* encapsulated with skim milk-alginate increased as much as 2.75±0. 02 and 1.61±0.04 log cycles, respectively.

(Author)

Key Words: Encapsulation, Extrusion, Probiotics, Cell Resilience, Viability

UDC: 613.281

Hafid, H. (Department of Animal Science, Universitas Halu Oleo)
Napirah, A. (Department of Animal Science, Universitas Halu Oleo)
Sarifu, S.M. (Department of Animal Science, Universitas Halu Oleo)
Rahman (Department of Animal Science, Universitas Halu Oleo)
Inderawati (Department of Animal Science, Universitas Halu Oleo)
Nuraini (Department of Animal Science, Universitas Halu Oleo)
Hasnudi (Departement of Animal Husbandry, Faculty of Animal Husbandry, Universitas Sumatera Utara)

Pengaruh stimulasi listrik pada sifat fisik dan organoleptik daging itik Muscovy (Effect of electrical stimulation on physical and organoleptic properties of Muscovy duck meat)

(Org: Eng)

JITV 23(4): 202-209

This research was aimed to study the effect of electrical stimulation period on physical and organoleptic properties of Muscovy duck meat. This research used 20 female Muscovy ducks, 1.5-2 years of age. The ducks were divided into 5 groups treatments for 4 replications. The treatments were period of electrical stimulation: 0, 5, 10, 15, and 20 minutes. The result showed that period of electrical stimulation did not affect ($P>0.05$) cooking loss but significantly affected ($P<0.05$) the tenderness, color, flavour, aroma, pH, and juiciness of duck meat. The best treatment was 20 minutes stimulation.

(Author)

Key Words: Muscovy Duck Meat, Electrical Stimulation, Physical properties, Organoleptic Properties

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.

, 2018

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.

, 2018

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 23 No. 4 2018).

1.	Prof. Dr. Endang Tri Margawati	:	Biotechnology - Research Center for Biotecnology, IIS
2.	Dr. Hartati	:	Animal Breeding and Genetics - Beef Cattle Research Station
3.	Prof. Dr. Iis Arifiantini	:	Reproduction and Pathology - Bogor Agricultural University
4.	Dr. Sri Yuliani, MT	:	Post-Harvest Technology - ICAPOSTRD
5.	Dr. Siti Darodjah Rasad	:	Animal Reproduction - Padjajaran University
6.	Prof. Dr. Abubakar	:	Post-Harvest Technology - ICAPOSTRD
7.	Prof. Dr. Ni Wayan Kurniani Karja	:	Biotechnology Reproduction - Bogor Agricultural University
8.	Dr. Tatan Kostaman	:	Physiology and Animal Reproduction - IRIAP
9.	Dr. Elizabeth Wina	:	Animal Feed and Nutrition - IRIAP

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 23, Number 4, December 2018 ISSN 0853-7380 E-ISSN 2252-696X

LIST OF CONTENT

Registered in: