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PREFACE

In this last edition , volume 22 no 4 December 2017 we proudly present articles from Breeding and Genetic, Reproduction Technology, Feed and Nutrition Technology, and Veterinary Science, those are: “Genetic polymorphisms of IGF1, GH, and OPN genes in crosses Peranakan Ongole cattle based on birth type in Central Java”; “Effect of Bali cattle ovarian status on oocytes nuclear maturation and in vitro fertilization rate”, “Supplementation of molasses and branched-chain amino acid to increase in vitro digestibility of ammoniated corn cob in ruminants feed”; “Growth and feed utilization of Boer x Kacang crossbred goats offered total mixed rations of different protein and energy levels”; “Anticoccidial activity of Artemisinin and extract of *Artemesia annua* leaves in chicken infected by *Eimeria tenella*” and “Contamination of *Escherichia coli* O157:H7 in dairy cow farms”.

Hopefully these articles would offer any benefit to readers and the end-users of technological innovation. We extent high appreciation to all authors and peer reviewers those contribute in this edition that would make this journal published. We hope other authors will contribute in the future.

Chief Editor;

Bogor, December 2017

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Genetic Polymorphisms of IGF1, GH, and OPN Genes in Crosses Peranakan Ongole Cattle Based on Birth Type in Central Java

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ABSTRAK

Anggraeni A, Talib C, Asmarasari SA, Herawati T, Andreas E. 2017. Polimerfisme genetik dari gen IGF1, GH dan OPN pada persilangan sapi PO berdasarkan tipe kelahiran di Jawa Tengah. JITV 22(4): 165-172. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1625

Polimorfisme genetik dari gen IGF1, GH, dan OPN pada sapi hasil persilangan Sapi Peranakan Ongole (PO) silangan Berdasarkan Tipe Kelahiran di Jawa Tengah. Perbaikan produktivitas dengan cara menyilangkan sapi PO terhadap sapi potong eksotik melalui kawin IB disukai banyak peternak di Jawa Tengah. Fertilitas menentukan kemampuan seekor induk sapi dalam menghasilkan anak. Gen IGF1 diduga mempengaruhi kelahiran kembar dan ganda pada sapi, sedangkan gen GH dan OPN menentukan kesuburan reproduksi. Polimorfisme genetik ketiga gen ini dipelajari pada sapi PO silangan untuk kelahiran tunggal (T) dan kelahiran (histori) kembar dan ganda (G) berasal dari dua kabupaten di Jawa Tengah, yaitu dari Sragen (T = 7 ekor, dan G = 13 ekor) dan dari Kendal (T = 9 ekor, dan G = 16 ekor). Polimorfisme genetik diidentifikasi dengan metoda PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) menggunakan enzim restriksi *Sna*BI (gen IGF1), *MSp*I (gen GH), dan *Bsr*I (gen OPN). Lokus IGF1|*Sna*BI dari sapi PO silangan yang diamati tidak memberikan varian SNP atau bersifat monomorfik untuk tipe kelahiran tunggal maupun kembar dan ganda. Sebaliknya lokus GH|*MSp*I dan OPN|*Bsr*I bersifat polimorfik dengan tingkat keragaman cukup tinggi. Disimpulkan lokus IGF1| *Sna*BI tidak bisa dipakai untuk melihat kemungkinan adanya kontrol genetik kelahiran kembar (ganda), tetapi lokus GH|*MSp*I dan OPN|*Bsr*I bisa dipertimbangkan sebagai informasi awal untuk seleksi molekular pada sifat kesuburan dari sapi potong.

Kata Kunci: Sapi Potong, Polimorfisme Genetik, Gen Kesuburan

ABSTRACT

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(4): 165-172. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1625

Genetic Polymorphisms of IGF1, GH, and OPN Genes in Crossbred of Peranakan Ongole Cattle Based on Birth Type in Central Java. Improved productivity by crossing Peranakan Ongole (PO) cattle to exotic beef cattle through AI mating is preferred by many farmers in Central Java. Fertility determines the ability of a cow to give birth. IGF1 gene is predicted to affect either twin or multiple births in cattle, whereas GH and OPN genes determine reproductive fertility. Genetic polymorphisms of these three genes were studied in the crossbred of PO cattle for single birth (S) as well as twin and multiple (M) births or their historical (M) ones from two districts in Central Java, from Sragen (S = 7 hd and M = 13 hd.) and from Kendal (S = 9 hd, and M = 16 hd.). Genetic polymorphisms were identified by PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) method using restriction enzymes of *Sna*BI (IGF1 gene), *MSp*I (GH gene), and *Bsr*I (OPN gene). IGF1|*Sna*BI locus in the observed crossbred PO did not have SNP polymorphism or monomorphic for single and twins or multiple births. In contrast, GH|*MSp*I and OPN|*Bsr*I loci were polymorphic with quite a high degree of diversity. In conclusion, the IGF1|*Sna*BI locus cannot be used to investigate possible genetic control of twins and multiple births, whilst GH|*MSp*I and OPN|*Bsr*I polymorphisms can be considered as initial information for selection on fertility traits in beef cattle.

Key Words: Beef Cattle, Genetic Polymorphism, Fertility Genes

INTRODUCTION

Beef cattle have a strategic role in producing red meat for the national population. Java Island is one of the centers of national beef cattle agribusiness, with the highest beef cattle population in the year 2016, as an example, are from East Java 4.407.807 hd., Central Java 674.573 hd., West Java 413.372 hd., and Yogyakarta Special Regency 309.018 hd respectively (DGLAH 2017). Central Java thus becomes one of the national beef cattle production. Animals are mostly raised by small farmers under a cow-calf operation (CCO) system. Crossbreeding through artificial insemination technique is preferred especially by farmers in many areas in Central Java. This is due to adequate facilities and human resources (inseminator) in supporting the easy implementation for artificial insemination (AI) mating. The crossing is preferred because farmers like calves to grow rapidly to give heavier weights, compared to those calves of local beef cattle. Crosses by AI mating are usually performed between local Peranakan Ongole (PO) cattle by the frozen semen of exotic beef cattle bulls mainly from *Bos taurus*, such as Simmental, Limousine, and Brahman.

The ability of reproduction of a cow to produce a calf within a short calving interval (around 12 months) is crucial for the success of beef cattle agribusiness. Another aspect that is also interesting to note, is to see the chances of twinning or multiple births in beef cattle. Large ruminant is very well known as uniparous species. It is very common for beef cattle to have a single calf in one birth (Komisarek & Dorynek 2002; Çobanoğlu 2010). However, at a low level, incidences of cows calving twins or multiple calves per birth occur. The natural frequency of twin and multiple parturitions in cattle ranges from 0.5-4% depending on many genetic and environmental factors (Sawa et al. 2012). Many studies have reported the cows of having twinning and multiple births without extra management and health services could cause detrimental for both cow and calves. Some negative effects from twin and multiple pregnancies were for decreasing fertility of cows by causing longer calving interval by 18 d., reproductive rest period by 9 d., and service period by 10 d., as well as increasing services per conception by 0.15 (Sawa et al. 2012). Twins and multiple births cause to lessen overall cow reproductive efficiency and productivity. However, twinning and multiple calves can give profitability under an intensive beef cattle breeding condition. Twinning or multiple births were possible to improve biological efficiency and production as a result of lower input costs during pregnancy and lactation cows (Karlsen et al. 2000).

Using molecular technology makes it possible to identify possible genes for having the control on twins and multiple births in beef cattle. Twins and multiple births in cows are known as a trait that follows a quantitative pattern, controlled by many genes. In addition, reproductive traits have many limitations for genetic improvement due to low heritability, sex-related expression, and difficult phenotypic measurements (Singh et al. 2014). The molecular selection at DNA level may help to get an earlier decision-making of animals to be considered as breeding stocks. Many studies have investigated genetic polymorphisms of major genes in relation to their effect on fertility. A number of growth and reproductive genes are predicted to affect twins and multiple births in cattle. Those genes are thought in affecting the number of ova produced and the number of transferable embryos, leading as potential gene markers for superovulation response (Yang et al. 2010; Deb et al. 2012; Singh et al. 2014).

Twinning rate in cattle is basically a sequential product of ovulation rate, conception rate, and embryo survival. Twinning and ovulation rates in cattle are strongly genetically correlated (0.75–0.9) (Gregory et al. 1997). This indicates the two traits are influenced by common loci (Lien et al. 2000). Certain variant SNPs or genotypes of IGF1 gene are presumed for having genetic control on twins and multiple births in beef cattle. A study by Echternkamp et al. (1990) proved that twinning and multiple pregnancies in cattle is associated by the increasing IGF-1 concentrations in both blood serum and follicular fluid. The IGF-1 stimulates mitogenesis of granulosa cells and steroidogenesis of ovarian cell cultures. This gene plays an important role in the regulation of folliculogenesis and may be involved in the process of multiple ovulations in cattle. IGF1 gene stimulates ovarian function by acting with gonadotropins to promote growth and steroidogenesis of ovarian cells (Lucy 2001). This is essential in stimulating multiple ovulation rates to give potential twins or multiple pregnancies during the process of fertilization.

GH and OPN genes are two of the other genes that play another role in maintaining twins and multiple pregnancies in cattle. Their roles are to give success on conception rate and embryo survival. Osteopontin (OPN) gene in cattle is located at chromosome 6 (BTA6) closed to quantitative trait loci (QTL) of fertility and milk production (Leonard et al. 2005). OPN is known as secreted phosphoprotein 1 (SPP1). OPN is expressed on maternal and foetal interface suggesting indicating that OPN gene has an important role in maintaining uterine-embryonic microenvironment (Johnson et al. 2003). This gene has the function to profoundly impact pregnancy, embryo implantation and placental development (Li et al. 2013). Whereas Growth Hormone (GH) gene plays a key role in postnatal growth and developments in tissue, muscle, bone, adipose tissue, udder gland (Sami et al. 2011). GH gene is involved in the processes of sexual differentiation and pubertal maturation as well as participated in gonadal steroidogenesis, gametogenesis and ovulation. This gene has additional roles in pregnancy and lactation through the actions as reflecting direct endocrine actions of pituitary GH or be mediated by its induction of hepatic or local IGF-I production (Hull & Harvey 2001). Significant relationships have been reported between GH concentrations and postpartum period and fertility of cows (Lucy 2008).

This study was aimed to investigate genetic polymorphisms of IGF1, OPN and GH genes in the crossbred PO cattle from two districts in Central Java Province. Information on genetic polymorphisms at certain DNA fragments of IGF1, OPN, and GH genes can be useful to investigate possible genetic control of twins and multiple births as well as fertility traits in beef cattle.

MATERIALS AND METHODS

Beef cattle as samples

Animals as samples in this study were crossed Peranakan Ongole (PO) cattle as the offsprings of crossbreeding PO females to exotic beef cattle breeds such as Simmental, Limousin, and Brahman. The crossed PO cattle were observed from Sragen Regency and Kendal Regency in Central Java Province. A female or a cow determined of having twins or multiple births was that cow for giving twins or multiple births at least once during her life, while that of historical twin or multiple births was that animal calved from twins or multiple births. Abbreviation of cows having (historical) twins or multiple births called M, whilst cow with single birth was grouped as control (S). A total number of the crossed PO cattle observed from Sragen amounted by 20 hd., consisting of the cows having twins or multiple births (M) by 13 hd. (9 females and 4 males), while those with single birth (S) by 7 hd. (all females). Further, the total number of the crossed PO cattle from Kendal were 25 hd, consisting the cows having twins or multiple births (M) by 16 hd (15 females, 1 male), while cows having single birth (S) by 9 hd. (4 females, 5 males).

Blood collection

All of those crossed PO cattle as samples were collected their fresh blood. Blood samples were taken for DNA analysis collected from jugular vein using a 21 G X 1 ½ sized vacutainer or 10 ml syringe containing heparin anticoagulant substance. A total of 1 to 2 ml of blood samples was stored in a 10 ml tube and added 8 ml of ethanol (EtOH) absolute containing 1 mM EDTA).

DNA extraction

DNA extraction was done by following four steps. 1. Sample preparation: 200 µl blood sample was inserted into 1.5 ml tube, added by distilled water 1000 µl, and centrifuged at 8000 rpm for 5 min. 2. Degradation of protein: 1xSTE sample was added to a solution of 350 µl, 40 µl SDS 10% and 10 µl proteinase K 5 mg/ml and incubated at 55°C for 2 hours. 3. Degradation of organic matter: the solution was added to 400 µl of phenol, 400 µl chloroform-isoamyl alcohol (24: 1) and 40 µl NaCl, then gently whisked at a room temperature for 1 hour. 4. DNA precipitation was performed by adding: 400 µl supernatant added 40 µl NaCl 5 M and 800 µl ethanol absolute, homogenized, frozen overnight.

DNA amplification

DNA genomes of the crossed PO cattle from single birth and twin (multiple) births were used as template in DNA amplification reaction (PCR reaction). The primer used for IGF1 gene, namely forward 5-CCTCTGCGGGGCTGAGTTGGT-3 and reverse 5-CGACTTGGCGGGCTTGAGAGGC-3 following Siadkowska et al. (2006). Primer used for Osteopontin (OPN) gene, namely forward GCAAATCAGAAGTGT GATAGAC-3, and reverse CCAAGCC AAACGTATGAGTT-3 following Leonard *et al.* (2005). While Growth Hormone (GH) gene used two pairs of primers, namely forward CCC ACG GGC AAG GC and GAG AAG; and reverse TGA GGA ACT GCA GGG GCC CA in accordance to Zhou et al. (2005).

PCR reaction

PCR reaction was performed with a total volume of 25 µl of solution mixture comprising Taq Polymerase DNA and 10X Taq Polymerase buffer (100 mM Tris-Cl, pH 8.3, 500mM KCL; 15mM $MgCl_2$; 0.01% gelatin); dNTP'S mix (dGTO, dATP, dTTP and dCTV) (Pharmacia); and sterile dH_2O. While PCR reaction conditions in the thermocycler engine were designed with pre-denaturation temperature by 93°C, denaturation by 94°C, annealing by 58-60°C, extension by 72°C, and post PCR 4°C. Cycles were repeated 33 times for multiplication.

Genotyping

To detect genetic variants of specific base fragments of each IGF1, OPN and GH genes were performed by PCR 5 µl product added 1 µl distillation water, 0.7 µl buffer, and restriction enzime 0.3 µl, then incubated at 37°C for 16 h. DNA cutting products were visualized on 2% agarose gel with 0.5 x TBE buffer (Tris-Borate EDTA, then coloured ethidium bromide. Electrophoretic results were observed with the aid of UV light transilluminator.

Analysis of genetic polymorphism

Analysis of genetic polymorphism or SNPs (single nucleotide polymorphisms) from each locus of IGF1|*Sna*BI GH|*MSp*I and OPN|*Bsr*I were conducted by Poggene 32 packet programme for:

a. Genotype and allele frequency by computing genotype number of all genotypes in a population
b. Hardy-Weinberg (H-W) Equilibrium by testing Chi-Square calculation
c. Heterozygosity observation (Ho) and expectation (He) by estimating on the base on allele frequency
d. Shannon's Information index

RESULTS AND DISCUSSION

Genotyping genes

SNP polymorphisms in IGF1 gene to be considered as potential molecular markers for economic traits providing reproduction traits (Leonard et al. 2005; Lucy 2008; Sami et al. 2011; Singh et al. 2014). DNA amplification of IGF1, OPN, and GH genes using PCR techniques by following specific primer design was successfully performed on all blood samples of the crossed PO cattle observed. Amplification products were obtained for single birth as well as twins and multiple births of the crossed PO cattle from Sragen (S = 7 hd, and M = 13 hd.) and also from Kendal (S = 9 hd, and M = 16 hd).

IGF1 gene

Bovine *IGF1* gene is located on chromosome 5 (BTA5) at nucleotide positions 66,532,877- 66,604,734 (UMD_3.1, Chromosome, *Bos taurus*, Ensembl) with the provisional nucleotide sequence is approximately 72 kb (ID number 281239) (Reyna et al. 2010). DNA amplification was performed at intron 1 of IGF1 gene that resulted in a DNA fragment size by 249 bp (Siadkowska et al. 2006**)**. Amplicon products were genotyped by *Sna*BI restriction enzyme. However, *Sna*BI enzyme did not cut specific DNA cutting sites at the intron 1 of IGF1 of all the crossed PO cattle observed. This happened both for single birth as well as for twins and multiple births from the two locations. Genotyping IGF1|*Sna*BI locus in the crossed PO cattle produced only one fragment of 249 bp. This means that all these animals had only one type of genotype, namely BB genotype. In contrast, there was not found either AA genotype (223 and 26 bp) or AB genotype (249, 223 and 26 bp). None of the genetic variation or SNP of IGF1|*Sna*BI locus was possibly due to no C/T base transition as a specific cutting site of *Sna*BI restriction enzyme.

OPN gene

Bovine OPN gene or *SPP1* gene comprises 7 exons spanning about 7 kb of genomic DNA (GenBank accession number NW 255516). OPN gene is composed of 6961 bp in total length: 1331 bp in processed length and 278 in protein product lengths. DNA amplicons of OPN gene were cut by a BsrI enzyme to detect a point mutation at specific base fragment at intron 4. Genetic polymorphism of OPN gene was studied by following Leonard et al. (2005) that found a C/T transition at intron 4 in a non-code 5 'region of OPN gene in Bos taurus cattle. Amplification products of DNA fragments of OPN|BsrI locus resulted in a base fragment size by 290 bp. For restricting DNA fragments of OPN|BsrI locus into two DNA bands by 200 and 90 bp was expressed as CC genotype. While, CT genotype was identified for the occurrence of three DNA bands by 290, 200, and 90 bp. If the DNA amplicons was not cut off by restriction enzyme resulting only one DNA band by 290 bp for TT genotype resulted.

GH gene

PCR products of GH gene were cut by the Msp1 enzyme to detect a point mutation at intron 3 of GH gene. When DNA fragments at intron 3 of GH gene after the cutting by the Msp1 enzyme produced two bands by 223 bp and 104 bp, so it was identified as BB genotype. For the producing only one DNA band by 327 bp was called AA genotype. When the existing three DNA bands of 327 bp, 223 pb, and 104 bp were named AB genotype. Results from this study were consistent to the previous study by Khatib et al. (2009**)** for identifying three AA, AB, and BB genotypes from the GH|Msp1 gene in Bos taurus dairy cattle.

Genotype and allele frequency

Genetic diversity is expressed as the total number of genetic characters that build the genetic makeup of a species, breed or population. Genetic diversity serves as a way for populations to adapt to changing environments. While more genetic variations exist, it is

Table 1. Genotype and allele frequencies of IGF1, OPN, and GH Genes of the crossed PO cattle based on birth type

Gene/	Single Birth					Twins / Multiple Births				
Location	Genotype			Allele		Genotype			Allele	
IGF1	AA	AB	BB	A	B	AA	AB	BB	A	B
Sragen	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000
Kendal	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000
Total	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000
OPN	CC	CT	TT	C	T	CC	CT	TT	C	T
Sragen	0.000	0.000	1.000	0.143	0.857	0.000	0.667	0.333	0.333	0.667
Kendal	0.000	0.250	0.750	0.214	0.786	0.000	0.471	0.529	0.300	0.700
Total	0.000	0.125	0.875	0.167	0.833	0.000	0.552	0.448	0.304	0.696
GH	AA	AB	BB	A	B	AA	AB	BB	A	B
Sragen	0.375	0.625	0.000	0.714	0.286	0.083	0.417	0.500	0.273	0.727
Kendal	0.000	0.000	1.000	0.000	1.000	0.118	0.471	0.412	0.344	0.656
Total	0.188	0.313	0.500	0.333	0.667	0.103	0.448	0.448	0.321	0.679

more likely that animals will have a better adaptation to the environment. Cattle is a diploid organism that has two potential alleles at a particular fragment of a gene. If a cattle have the same allele, it is expressed as homozygous, otherwise to be heterozygous. A codominant allele is very useful to serve as a genetic marker. The frequency of alleles and genotypes can reflect genetic diversity of animal population.

As previously stated that genotyping intron 1 of IGF1|SnaBI locus on all of the crossed PO cattle in this study produced the only BB genotype with the DNA fragment of 249 bp. This means each individual of both historical twin (multiple) births and the single birth of the crossed PO cattle from the two locations had the only BB genotype (Table 1). Thus only the B allele was found (1,000), otherwise no A allele. An allele is said to be monomorphic if that allele frequency is more than 0.99. No genetic variation or monomorphic of the specific base cutting site, known as C/T base transition, at intron 1 of IGF1|SnaBI locus was identified. Certain SNPs or genotypes of IGF1 gene are presumed for having genetic control in stimulating multiple ovulation rates to give potential twins or multiple pregnancies (Echternkamp et al. 1990; Gregory et al. 1997; Singh et al. 2014). By the finding monomorphic base at the specific base of SnaBI cutting site from this results indicated that IGF1|*Sna*BI locus cannot be used to investigate possible genetic control of twins and multiple births in the crossbred PO cattle.

Several studies, however, identified an SNP at the intron 1 of IGF1 using a *Sna*BI restriction enzyme showing a T>C transition at the specific cutting site of *Sna*BI restriction enzyme in Cattle. Frequently B allele was reported dominant than A allele showing that BB cattle very dominant to those AA and AB cattle. Yurnalis et al. (2017) from a study in local Pesisir cattle in West Sumatera reported frequencies of AA, AB, and BB genotypes were successively 0.0125, 0.0125, and 0.9750 and resulted frequencies of A and B alleles were 0.00625, and 0.99375 respectively. Genetic polymorphism of IGF-I gene was also reported from Bali cattle of which an SNP as a transition mutation T/C being identified by restriction enzyme *Rsa*I at exon 4 with the frequency of C allele higher than that of T allele, namely 0.836 vs. 0.164 (Maskura et al. 2012). Furtherly genotyping IGF1 by PCR – RFLP method using *Taq*I and *Msp*I restriction enzymes and amplification-created restriction site (ACRS) one by *Sna*BI, *Tas*I and *Taq*I restriction enzymes in Poland Cattle, Szewczuk (2016) reported frequencies of the most common alleles by 0.67 for T allele (IGF1|*Sna*BI), 0.85 for A allele (IGF1|*Tas*I), 0.95 for C allele (IGF1R|*Taq*I), 0.84 for G allele (IGF1R|*Msp*I) and 0.69 for G allele (IGF1R|*Taq*I).

Genotyping at intron 4 of OPN|BsrI polymorphism in the crossed PO cattle did not produce CC genotype for both single and twins or multiple births for the two locations. Single birth cattle in both locations had higher frequencies of TT genotype (0.750 to 1.000) than CT genotype (0.000 - 0.250). Whilst for twins and multiple births ones had higher frequencies of CT genotype (0.471 - 0.667) than TT one (0.333 - 0.529). These results suggested that single birth had more common for homozygous BB cattle, whereas twins and multiple births were more frequent in heterozygous CT cattle. High CT and TT genotypes resulted in higher T allele against C allele for both single birth or twins and multiple births of the crossed PO cattle in this study. Pareek et al. (2008) reported OPN or *SPP1 C>T* SNP polymorphism was also found within the intron IV of bovines *SPP1* gene that was investigated in selected breeds of beef (Hereford and Limousine) and dairy (Polish HF and Polish Red) cattle. The study revealed significant differences in the distribution of *C* and *T* alleles among the analysed panel of cattle breeds. In general allele frequencies of T and C were 0.59 and 0.41 respectively; so frequencies of TT, CT and CC genotypes were 34.69, 48.62, and 16.69 respectively.

Growth hormone (GH) is an anabolic hormone synthesized and secreted by somatotropic cells in an anterior pituitary lobe (Ayuk & Sheppard 2006). The specific DNA cutting site at intron 3 of GH|MspI locus lies at position 1547 (Zhang et al. 1993). Genotyping DNA fragments at intron 3 of GH|MspI SNP polymorphism in the crossed PO cattle in twin and multiple births had higher frequencies of AB genotype (0.417 - 0.471) and BB genotype (0.412 - 0.500) than AA genotype (0.083 - 0.118). The same pattern was found in single birth type. AB cattle were found higher than AA one. Even in Kendal, single birth cattle was just identified only for BB genotype. These results indicated that both single birth and twins (multiple) births of the crossed PO cattle had higher AB and BB genotype than AA one. Thus frequencies of B allele were higher than those of A allele A across birth type and location. DNA polymorphism of GH|MspI locus in Aceh cattle was reported by Putra et al. (2013) to have only TT genotype (BB genotype) resulting frequency of T allele was 1,000 and monomorphic.

Heterozygosity

Estimation of heterozygosity value was obtained to determine the level of genetic diversity in the crossed PO cattle population observed. This information can be used to help the effectiveness of selection program on breeding stocks to be used as a genetic source in the next generation (Marson et al. 2005). Heterozygosity observation (Ho) of OPN|BsrI and GH|MspI loci of the crossed PO cattle by birth type are listed in Table 2. Heterozygosity expectation (He) is required to know the difference to heterozygosity observation (Ho). For the IGF1|SnaBI locus with the only BB genotype in all of the crossed PO cattle lead to Ho value of 0.000.

For OPN|BsrI locus in the crossed PO cattle from both locations showed Ho values were higher than He values. It was for single birth namely Ho = 0.2857 - 0.4286 vs He = 0.2637 - 0.3626; while that for multiple births, namely Ho = 0.6000 - 0.6667 vs He = 0.4345 - 0.4638. Ho values were higher than the He values from this OPN|BsrI locus could be an indication for a good heterozygosity level this gene polymorphism in the crossed PO cattle either for single birth and twins or multiple births. OPN|BsrI locus, therefore, could be considered as a molecular selection in regarding increase probability of twins or multiple births in beef cattle. According to Javanmard et al. (2005), a heterozygosity value above 0.5 (50%) indicates a high variation of a gene in a population. Molecular selection by using OPN|BsrI gene might be possible to give a good selection respond in improving twin or multiple births in beef cattle.

However, the opposite result was found to GH|MspI locus in which the crossed PO cattle in multiple births had relatively smaller Ho values compared to He values, ie Ho = 0.3636 - 0.4375 vs He = 0.4156 - 0.4657. This indicated heterozygosity level of GH|MspI

Table 2. Heterozygosity observation (Ho) and heterozygosity expectation (He) of OPN and GH genes of the crossed Peranakan Ongole based on birth type

Gene	Location	Single birth			Twins / Multiple Births		
	Heterozygosity	Ho	He	Polymor.	Ho	He	Polymor
OPN	Sragen	0.2857	0.2637	100 %	0.6667	0.4638	100 %
	Kendal	0.4286	0.3626	100 %	0.6000	0.4345	100 %
	Total	0.3333	0.2874	100 %	0.6071	0.4305	100 %
GH	Sragen	0.5714	0.4396	100 %	0.3636	0.4156	100 %
	Kendal	0.0000	0.0000	0 %	0.4375	0.4657	100 %
	Total	0.2667	0.4598	100 %	0.4286	0.4442	100 %

Tabel 3. Hardy-Weinberg Equilibrium of OPN and GH genes of the crossed Ongole Grade cattle based on birth type

Gene	Location	Single Birth			Twins/Multiple Births		
	H-W Equi.	χ^2	$\chi^2_{(0,05)}$	Signif.	χ^2	$\chi^2_{(0,05)}$	Signif.
OPN	Sragen	0.9009	0.7630	*	2.5667	0.1091	*
	Kendal	0.2143	0.7857	ns	2.4000	0.1213	*
	Total	0.1667	0.8333	ns	4.9555	0.0260	*
GH	Sragen	0.8000	0.3711	*	0.2000	0.6547	ns
	Kendal	-	-	-	0.0636	0.8008	ns
	Total	2.8884	0.08922	*	0.0362	0.8492	ns

*) values in the same row shows significantly difference (P<0.05)

locus was relatively low in twin and multiple births in both locations. Generally, OPN|BsrI and GH|MspI loci.had high polymorphic level (100%), the exception was for GH|MspI for the single birth type from Kendal which was monomorphic with only the BB genotype

Hardy-weinberg equilibrium

Chi-square analysis can be used to determine whether or not the genotype frequency (p^2, 2pq, q^2) or allele frequencies (p and q) in a population. The results of Chi-square analysis can be used as an indicator of population equilibrium known as Hardy - Weinberg Equilibrium. Chi-square value ($\chi 2$) of OPN|BsrI locus in the crossed PO cattle showed significantly different ($\chi 2>\chi 2$ (0.05)) for single and multiple births in both locations, excepting for single birth in Kendal. It can be stated that allele frequency of OPN|BsrI locus of the crossed PO cattle was not in equilibrium for twins and multiple births from both locations.

$\chi 2$ value of the crossed PO cattle of GH|MspI locus from single birth type could not calculate because of it was monomorphic. Genotype frequencies in the crossed PO cattle, therefore, did not meet the assumptions for Hardy-Weinberg equilibrium analysis. Furthermore, GH|MspI polymorphism in multiple births in both locations had Chi-Square values higher (($\chi 2>\chi 2$ (0.05)), so it can be stated that these two populations were in Hardy – Weinberg equilibrium. This means that mating among individual cattle from twins and multiple births in both locations was in equilibrium. A population can be stated in a Hardy-Weinberg equilibrium if the frequency of dominant and recessive alleles are constant from generation to generation, there is no selection, mutation, migration, and genetic drift.

CONCLUSION

Genotyping intron 1 of IGF1|SnaBI locus on all of the crossed PO cattle in this study produced the only BB genotype with the DNA fragment of 249 bp. By the finding monomorphic base at the specific base of SnaBI cutting site from this results indicated that IGF1|*Sna*BI locus cannot be used to investigate possible genetic control of twins and multiple births in the crossbred PO cattle. Whilst genotyping GH|*MSp*I and OPN|*Bsr*I loci were polymorphic that useful as initial information for molecular selection on fertility traits in beef cattle. GH|MspI locus in the crossed PO cattle from multiple births was in equilibrium H-W equilibrium contrasting to OPN|BsrI locus indicating that the frequencies of AA and BB alleles of GH|MspI were constant by generations.

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Effect of Bali Cattle Ovarian Status on Oocytes Nuclear Maturation and *In Vitro* Fertilization Rate

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ABSTRAK

Sonjaya H, Yusuf M, Hamdana A, Utamy RF, Gustina S, Hasbi H. 2017. Pengaruh status ovarium sapi Bali terhadap maturasi inti oosit dan tingkat fertilitas *in vitro*. JITV 22(4): 173-178. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1585

Penelitian ini dilakukan untuk mengetahui pengaruh status reproduksi ovarium yang ditandai dengan keberadaan corpus luteum (CL) dan folikel dominan (FD) terhadap tingkat pematangan inti dan fertilisasi oosit sapi bali in vitro. Pasangan ovarium dari rumah potong hewan (RPH) dipisahkan menjadi empat kelompok, yaitu: 1) ovarium dengan CL dan FD, 2) ovarium tanpa CL dan ada FD, 3) ovarium dengan CL dan tanpa CL, dan 4) ovarium tanpa CL dan FD. Penelitian ini dibagi dalam dua tahap. Tahap I, oosit dikoleksi dengan teknik pencacahan dalam medium Phosphate Buffer Saline (PBS) yang disuplementasi dengan Fetal Bovine Serum (FBS) 10% dan penicilin streptomycin 100 IU/ml. Oosit dimatangkan selama 24 jam, 38,5°C dalam inkubator CO2 5%, menggunakan medium TCM-199 yang disuplementasi dengan FBS 10%, Follicle Stimulating Hormone (FSH) 10 IU/ml, Luteinizing Hormone (LH) 10 IU/ml, dan gentamycin 50 µg/ml. Tahap II, oosit dimatangan dalam media seperti pada penelitian tahap I, kemudian difertilisasi secara in vitro untuk mengamati pembentukan pronukleus. Hasil penelitian tahap I menunjukkan bahwa jumlah oosit yang mencapai fase metaphase II (MII) lebih tinggi (P<0,01) pada pasangan ovarium ada CL dan tanpa FD (89,47%) dibandingkan dengan tanpa CL dan tanpa FD (75,47%), tanpa CL dan ada FD (74,41%), dan ada CL dan ada FD (65,52%). Status reproduksi ovarium yang memiliki CL tetapi tanpa FD memiliki jumlah oosit yang mencapai tahap MII tertinggi. Penelitian tahap II menunjukkan bahwa status reproduksi ovarium tidak berpengaruh terhadap tingkat fertilisasi oosit sapi bali. Hasil penelitian menyimpulkan bahwa persentase oosit yang mencapai tahap MII dari ovarium yang memiliki CL dan tidak memiliki FD tertinggi, namun tingkat fertilisasi tidak dipengaruhi oleh status reproduksi ovarium.

Kata Kunci: Status Ovarium, Oosit, Sapi Bali, Pematangan, Fertilisasi

ABSTRACT

Sonjaya H, Yusuf M, Hamdana A, Utamy RF, Gustina S, Hasbi H. 2017. Effect of Bali cattle ovarian status on oocytes nuclear maturation and *in vitro* fertilization rate. JITV 22(4): 173-178. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1585

The aim of this study was to investigate whether the reproductive status influences the nuclear maturation and fertilization rates of bali cattle oocytes in vitro. Several pairs of ovary were classified into four groups: 1) ovaries with Corpus Luteum (CL) and Dominant Follicle (DF), 2) ovaries without CL and with DF, 3) ovaries with CL and without DF, 4) ovaries without both CL and DF. In the first experiment, oocytes were collected by slicing method in Phosphate Buffer Saline (PBS) medium supplemented with 10% Fetal Bovine Serum (FBS) and 100 IU/ml penicillin streptomycin. Oocytes were matured in tissue culture medium (TCM)-199 supplemented with 10% Fetal Bovine Serum (FBS), 10 IU/ml Follicle Stimulating Hormone (FSH), 10 IU/ml Luteinizing Hormone (LH), and 50 µg/ml gentamycin. Oocytes were matured in 5% CO2 incubator, 38ºC for 24 h. In the second experiment, oocytes were matured and then fertilized in vitro to observe pronuclear formation. The first experiment showed that the percentage of oocytes reached methaphase-II (MII) stage on ovaries with CL and without DF (89.47%) were higher (P<0,01) compared to ovaries without both CL and DF (75,47%), ovaries without CL and with DF (74.,41%), or ovaries with CL and DF (65,52%). The result of second experiment showed that the ovarian reproductive status was not significantly different (P>0.05) on fertilization rate.

Key Words: Ovarian Status, Oocytes, Bali Cattle, Maturation, Fertilization

INTRODUCTION

Embryo production in vitro (EPIV) is one of the *assisted reproductive technology* (ART) consisted of *in vitro maturation* (IVM), *in vitro fertilization* (IVF) and *in vitro culture* (IVC) (Rahman et al. 2008, Hegab et al. 2009). Genetic material of the animal with abnormal reproduction function may be rescued by this EPIV technology.

The success of the EPIV technology is determined by many factors, such as oocytes source used. Oocytes grow in the follicle environment in the ovary following certain growth circle. Follicle growth wave consists of recruitment phase, selection, dominant and some have atresia (Campanile et al. 2010). In cattle and sheep, it is possible for several follicle waves in one estrus circle, generally two to three times in sheep which each may produce more than dominant follicle (DF) (Evans 2003). This dominant follicle existence will decrease FSH concentration (Gonzalez-Bulnes et al. 2004) and leading a pressure to other follicle growths in the same wave causing a regression (Varishaga et al. 1998). Then, the DF will ovulate if there is no corpus lutheum (CL). Remnant of ovulated DF forms CL. This CL consists of cells that will produce progesterone hormone useful in implantation process and pregnancy maintenance. The aim of this study was to investigate whether the reproductive status influences the nuclear maturation and fertilization rates of bali cattle oocytes *in vitro*.

MATERIALS AND METHODS

Stage I. Oocytes collection and maturation *In Vitro*

Ovarium of bali cattle were collected from a slaughterhouse and transported to the laboratory in the solution of 0.9% NaCl supplemented with 100 IU/ml penicilin and 100 µg/ml *streptomycin sulfate*. Oocyte collection was performed by aspiration technique using a 18-G syringe. Phosphate buffered saline (PBS) supplemented with 10% *fetal bovine serum* (FBS) (Sigma, USA) was used as the collection medium. Oocytes with homogenous cytoplasm and surrounded by ≥3 layers of cumulus cells were selected under a stereo-microscope and were graded into either A or B (Abdoon et al. 2014). The selected oocytes were washed three times in the maturation media, then the maturation was performed in the *tissue culture medium* (TCM) 199 (Sigma, USA) supplemented with 10 IU/ml *pregnant mare serum gonadotrophin* (PMSG) (Intergonan, Intervet Deutschland GmbH), 10 IU/ml *human chorionic gonadotrophin* (hCG) (Chorulon, Intervet international B.V. Boxmeer-Holland), 1 µg/ml *estradiol* (Intervet international B.V. Boxmeer-Holland) and 50 µg/ml *gentamycin* (Sigma, USA). The oocyte maturation was performed in a 35-mm Petri dish (Nunclon, Denmark) in the form of drop, 50 µl in volume, containing 10-15 oocytes per drop, covered with mineral oil (Sigma, USA). The oocytes were incubated in a 5% CO_2, 38.5°C incubator for 24 hours (modified from Hasbi et al. 2017).

Evaluation of nucleus maturation level and the staining process

Evaluation of nucleus maturation level was assessed on the basis of meiosis from *germinal vesicle* (GV) to *metaphase* II (MII) on 2%-aceto-orcein staining. *Germinal vesicle* stage was characterised by a clearly visible nucleus and its membrane. *Germinal vesicle breakdown* (GVBD) was marked by the fragmentation of the nucleus membrane and indistict nucleus. Metaphase I (MI) was marked by the existence of homolog chromosomes lined up in the equator, whereas in the anaphase I (AI) stage, the chromosomes were moved to both poles. In the telophase I (TI) stage, the chromosomes had reached both poles. Metaphase II (MII) was marked by polar body I and the same chromosome structure with the one in the MI stage (Shirazi & Sadeghi 2007). Nucleus maturation level was determined on the basis of oocytes percentage able to reach the MII (Bijttebier et al. 2008).

After 24 hours maturation, the cumulus cells surrounding the oocytes were removed using 0.25 % *hyaluronidase* (Sigma, USA) followed by denotation by repeated pipetting using a 110-120 µm syringe (according to oocyte size). The oocytes were suspended in 0.7 % KCl and placed as a drop on the cover glass with paraffin and vaseline pads in its four corners. The cover glass was placed upside down on an object glass. The oocytes were fixed by dipping the glasses into fixation solution consisting of acetic acid and ethanol (1 : 3) for 3 days. The cells were dehydrated by soaking in ethanol, then stained with 2% aceto-prcein for 5 minutes. The dye was cleaned using 25% acetate acid and the four corners of cover glass was smeared by clear nail polish. Then, the morphology of the oocytes was performed under microscope (Zeiss Axio Imager A2) using Zeiss Axiocam HRc.

Stage II. *In Vitro* fertilization

Collection and maturation of oocytes followed the same procedure as in the stage I. Frozen semen was thawed in 37°C for 20 second and then centrifuged at 700 G for 5 minutes in the fertilization media (Suzuki et al. 2000). After the centrifugation, sperm sediment was suspended in fertilization media to a concentration of 1.5×10^6 spermatozoa/ml. After the IVM, the oocytes were washed in fertilization media twice, then moved into drop fertilization media (10-15 oocytes in 100 µl fertilization media) and covered with mineral oil (Sigma-Aldrich. Inc, M-8410) followed by incubation at 38.5°C for 16 hours in 5% CO_2 incubator (modified from Hasbi et al. 2017). Fertilization level was evaluated in a basis of pronucleus formation with 2% *aceto-orcein* staining as in stage 1.

Data analysis

Data were presented in the form of percentage. Differences between treatments were analyzed using ANOVA. In case significant differences were found, the smallest real test was carried out. All statistical analyses were carried out using SPSS 17 (SPSS^{Inc}).

RESULTS AND DISCUSSION

Maturation stage of oocytes nucleus

Oocyte in vitro maturation stage is detemined by quality of the oocytes used. In this study, cumulus cells were formed after in vitro maturation for 24 hours (Figure 1B). Rahman et al. (2008) reported that formation of cumulus cells is followed by changes in the gap junction consisted of *transmembrane channels*. The latter were formed by hexamer proteins including connective proteins. In horse, cattle, sheep and mouse, the cumulus cells have about 43 connective proteins. The existence of cumulus cells played a crucial role in the protein synthesis process before the *germinal vesicle breakdown* (GVBD). In sheep and cattle oocytes, the GVBD functions as supply of nutrient, energy substrate, messenger molecules for oocytes development and hormone effect in the *cumulus oocyte complexes* (COCs) (Rahman et al. 2008).

The communication between oocytes and cumulus cells plays crucial role in future maturation and development process of oocytes during IVC. Tthe communication is connected by gap junctional communication (GJC), where the components activate the meiosis process, glutathione or substrate glutamine and cysteine, a small regulator molecule lighter than 1 kDa, substrat molecule with lighter than 1,200 Da such as Ca^{2+}; nucleotide and amino acids are transferred from cumulus cells through the GJC (Shirazi et al. 2007).

This study showed that the most matured oocytes have started the meiosis stage indicated by the change of nuclear oocytes from GV to MII stages (Figure 1C-F). The number of oocytes reached MII stage based on the ovarium reproduction status was presented in the Table 1.

This study showed that reproduction status of ovary was significantly ($P<0.01$) influenced the number of oocyte of bali cattle that reached metaphase II (MII) stage. The statistic test showed reproduction status of ovary +CL –FD (89.47%) had more ($P<0.01$) oocytes reached MII than the +CL +FD (65.52%), -CL +FD (74.41%), and -CL –FD (75.47%). Whereas, the number of oocytes reached MII stage in the ovary -CL +FD was not different ($P>0.05$) with the ovary -CL –FD, but significantly higher than the +CL +FD. The existence of CL and DF on the ovarian couple affects in vitro maturation stage of oocyte nucleus of bali cattle.

Generally, maturation stage of oocytes nucleus from ovarian couple that have CL and DF tends to be lower. This showed that progesterone from the CL and inhibin from the DF gives a negative effect to in vitro maturation process of nucleus. Ooe et al. (1997) reported that in the cattle, ultrasonography use and FSH hormone administration showed that the number of follicle, the number of aspirated oocyte, the number of life oocyte which then experiencing division and forming blastocyst in vitro; the lowest was in the animal with DF compared to the animal without DF. This FSH administration on the animal with DF did not give positive response to subordinate follicle development. In vice versa, Boediono & Setiadi (2006) stated that CL existence in the ovary has positive correlation with the number of grow follicle. Progesterone produced from CL may inhibit DF growth and remove inhibin effect, so that subordinate follicle is able to grow (Kor 2014). Varishaga et al. (1998) reported that the highest number of collected oocyte, division and blastocyst level was obtained from the ovary with CL and without DF followed by oocyte from ovary with CL and DF. Then, Vassena et al. (2003) reported that there was no significant difference in blastocyst level result of cattle in basis of the difference of reproduction status of ovary marked by CL and DF existence. Even though, generally the blastocyst level continue to rise on the ovary with CL. This is allegedly that CL produced progesterone plays a role to conquer negative effect inflicted by inhibin existence. High A inhibin on the follicle will drive to FSH secretion and another follicles development on the cattle, sheep and buffalo (Perera 2011).

In Vitro **fertilization level of oocytes**

Fertilization level was assessed by counting the number of oocyte forming two or more pronucleus (Figure 2).

This study showed that reproduction status of ovary did not significantly affect ($P>0.05$) the number of fertilized oocytes (forming two or more pronucleus). This study also revealed a higher fertility level (60.29%) on the oocyte from ovary without CL and DF than other ovary reproductive status (Table 2). The quality of oocyte used in this study was uniform with the homogenous cytoplasm surrounded by compact cumulus cells indicating a uniform development. This study also provided an indication that inhibit supposedly produced by DF may not affect in vitro fertility level of bali cattle oocytes.

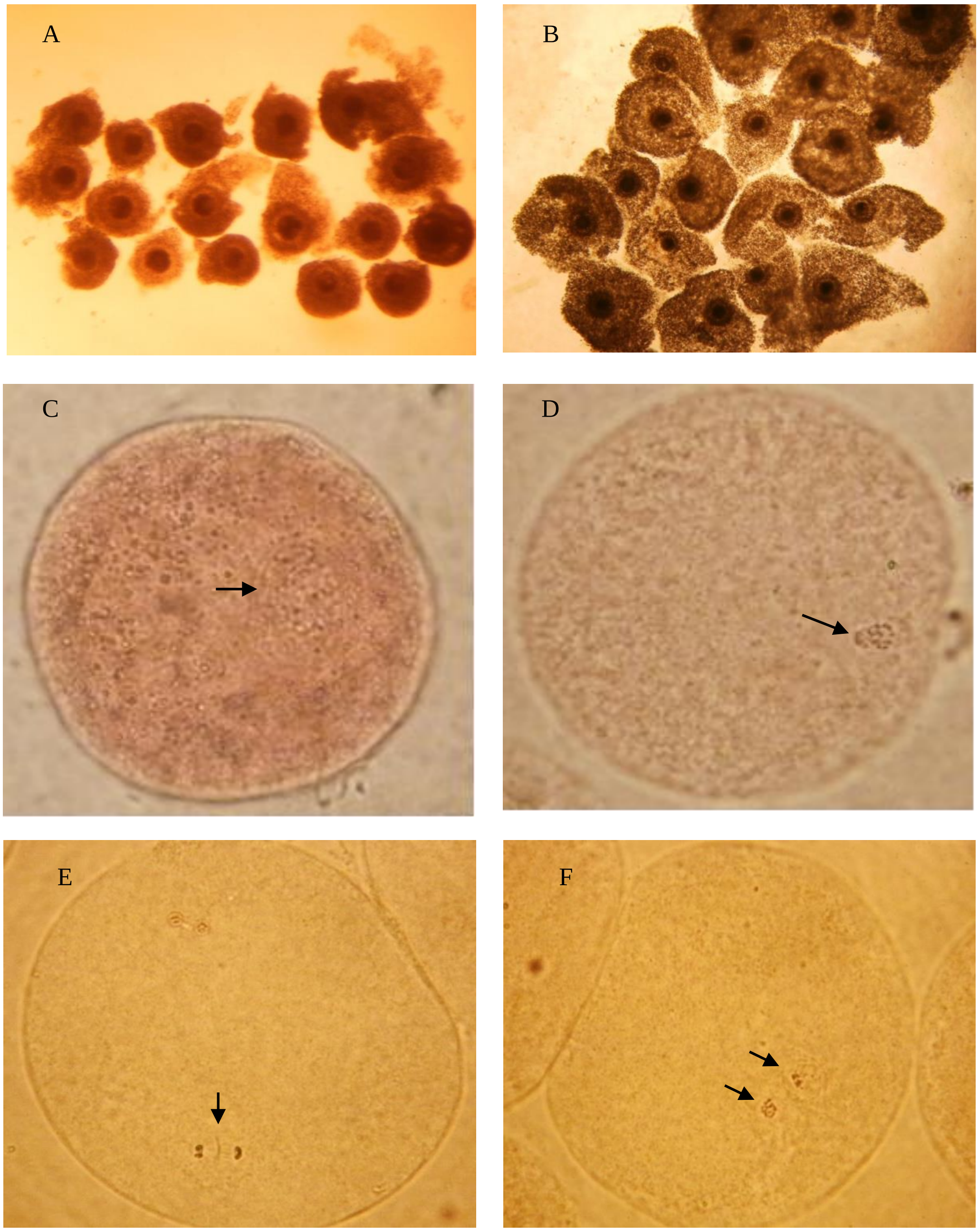

Figure 1. The development of bali-cattle oocyte. A. Oocyte before maturation (200 x magnification), B. Oocyte after maturation (200 x magnification), O: Oocyte (SK: cumulus cells, and ESK: cumulus cell expansion), C. *Germinal Vesicle* (GV), D. *Metaphase* I (MI), E. Anaphase/Telophase (A/T), and F. *Metaphase* II (MII) (the arrows).

The success of in vitro fertilization is highly influenced by oocyte source used. The criterion of oocytes used in this study was homogenous cytoplasm surrounded by compact cumulus cells. Those oocytes were expected from tertiary follicle that have the ability to start the meiosis process. Oocytes from tertiary follicle is reported to produce *reactive oxygen species* (ROS) component, antioxidant, hormone, metabolite (Hennet & Combelles 2012), glucose, pyruvate, and glycine (Pinero-Sagredo et al. 2010) playing crucial roles to promote fertility process.

The meiosis process is started by activating G protein that activated *phospholipase* C, which in turn hydrolyzed *phosphoinositide* to form inositol

Table 1. Maturation stage of cattle oocyte nucleus from ovary with different reproduction status

Reproduction Status of Ovary	The number of oocytes	Maturation Stage of Nucleus (%)				
		GV	GVBD	MI	AI/TI	MII
+CL +FD	58	1 (1.72)	0 (0.00)	19 (32.76)	0 (0.00)	38 (65.52)^{a}
-CL +FD	43	1 (2.33)	0 (0.00)	9 (20.93)	1 (2.33)	32 (74.41)^{b}
+CL -FD	57	0 (0.00)	0 (0.00)	6 (10.53)	0 (0.00)	51 (89.47)^{c}
-CL -FD	53	0 (0.00)	0 (0.00)	13 (24.53)	0 (0.00)	40 (75.47)^{b}

Description: +CL: CL present, -CL: CL absent CL, +FD DF present:, -FD: DF absent, GV: *germinal vesicle*, GVBD: *germinal vesicle breakdown*, MI: *metaphase* I, AI-TI: *anaphase* I/*telophase* I, MII: *metaphase* II. Different superscript in the same column means significant different (P<0.01).

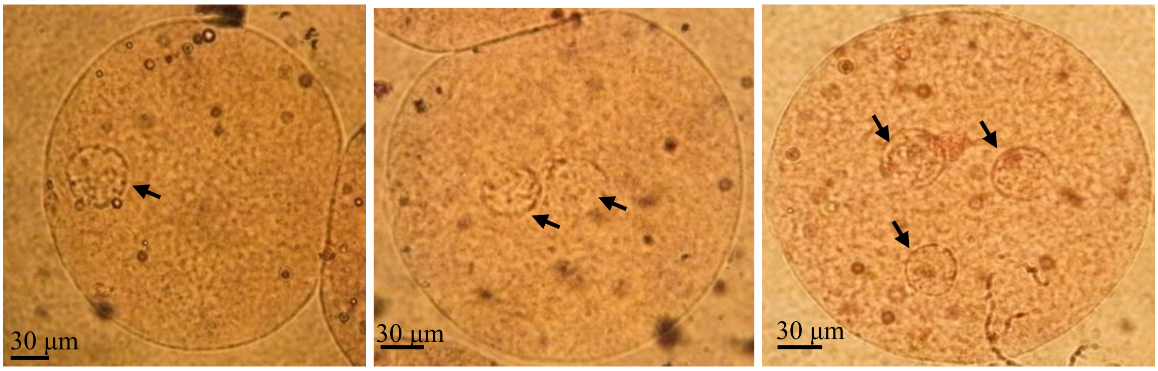

Figure 2. Forming of pronucleus after in vitro fertilization process: A. one pronucleus, B. two pronucleuses, C. three pronucleuses (the arrow).

Table 2. Fertilization level of bali cattle oocytes from ovarium with different reproduction status

Reproduction status of ovary	The number of oocyte	Forming of pronucleus				Fertilization level (%)
		0 PN (%)	1PN (%)	2 PN (%)	>2 PN (%)	
+CL +FD	47	3 (6.38)	17 (36.17)	14 (29.79)	13 (27.66)	27 (57.44)
-CL +FD	41	9 (21.95)	11 (26.83)	12 (29.27)	9 (21.95)	21 (51.21)
+CL –FD	58	22 (37.93)	17 (29.31)	10 (17.24)	9 (15.52)	19 (32.76)
-CL –FD	68	2 (2.94)	25 (36.76)	22 (32.35)	19 (27.94)	41 (60.29)

Description: PN: Pronucleus.

triphosphate mobilizing intracellular Ca^{2+} followed by influx of extracellular Ca^{2+} (Ajduk et al. 2008). Influx extracellular Ca^{2+} may inhibit *adenylyl cyclase* leading to decrease of cAMP/PKA and also may activate *calmoduline-dependent protein kinase* (CaM II kinase) which will modify or activate the *maturation promoting factor* (MPF) (Oh et al. 2010; Conti et al. 2012). Brevini et al. (2007) reported that distribution of organelle on the cytoplasm is closely related to the oocyte maturation and competition. Consequently, maturation of imperfect cytoplasm from the oocyte reached MII stage was allegedly to lead to low success of in vitro embryo production (Blanco et al. 2011).

CONCLUSION

The number of oocytes from ovarium of bali cattle with corpus luteum (+CL) and and without dominant follicle (–FD) reached metaphase-II stage (MII) was higher compared to the oocytes from other ovarium (+CL,+FD; -CL,+FD or -CD, -CD). However, reproduction status of ovarium did not affect the fertility level.

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Supplementation of Molasses and Branched-Chain Amino Acids to Increase *In Vitro* Digestibility of Ammoniated Corn Cob in Ruminants Feed

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ABSTRAK

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Tongkol jagung memiliki kandungan serat kasar dan lignin yang tinggi sehingga menyebabkan kecernaan nutriennya rendah. Penelitian bertujuan untuk meningkatkan kecernaan tongkol jagung (TJ) amoniasi melalui suplementasi molases dan asam amino bercabang (BCAA) valin, leusin dan isoleusin. Tongkol jagung diolah dengan penambahan urea 3%. Uji *in vitro* tahap pertama dilakukan dengan 4 taraf molases 0, 5, 10 dan 15% dari bahan kering (BK) TJ. Percobaan dilakukan dengan RAL 4 perlakuan yang diulang 4 kali. Uji *in vitro* tahap kedua, suplementasi valin = V, leusin = L dan isoleusin = I dengan taraf masing-masing 0,1 dan 0,2% dari BK TJ amoniasi. Terdapat 8 perlakuan yaitu A = V0,1 L0,1 I0,1; E = V0,2 L0,1 I0,1; B = V0,1 L0,1 I0,2; F = V0,2 L0,1 I0,2; C = V0,1 L0,2 I0,1; G = V0,2 L0,2 I0,1; D = V0,1 L0,2 I0,2; H = V0,2 L0,2 I0,2 dan 1 kontrol (V0,0 L0,0 I0,0). Percobaan dilakukan dengan RAL 9 perlakuan dan diulang 3 kali. Hasil percobaan menunjukkan bahwa pengolahan TJ dengan urea mampu meningkatkan kadar protein sebesar 78% (3,34% menjadi 5,95%) sedangkan NDF menurun sebesar >15,4%, ADF sebesar 7,9% dan lignin 16,7%. Nilai kecernaan BK TJ amoniasi meningkat (P<0,05) sebesar 7,5% (41,9 *vs* 43,51-46,26%) dan NDF sebesar 17,7% (38,41 *vs* 43,76-46,31%) karena tambahan molases. Suplementasi BCAA menghasilkan kecernaan BK, BO dan NDF tertinggi (P<0,05) pada perlakuan A, C, D dan G. Kecernaan BK, BO dan NDF pada perlakuan G dibandingkan dengan I masing-masing sebesar 31,4%, 27,5% dan 36,5% serta menghasilkan populasi bakteri total rumen tertinggi sebesar 12,4 x 10^9 koloni/ml. Dapat disimpulkan bahwa kecernaan TJ amoniasi meningkat melalui suplementasi molases 5% dan kombinasi BCAA yang terdiri dari valin 0,2%, leusin 0,2% dan isoleusin 0,1%.

Kata Kunci: Tongkol Jagung, Amoniasi, Molases, Asam Amino Bercabang

ABSTRACT

Puastuti W, Yulistiani D, Handiwirawan E. 2017. Supplementation of molasses and branched-chain amino acid to increase *in vitro* digestibility of ammoniated corn cob in ruminants feed. JITV 22(4): 179-187. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1664

Corn cob contains high fiber and lignin which causes low nutritive value. The objective of the study was to improve the digestibility of ammoniated corn cob (CC) by supplementation of molasses and branched-chain amino acid (BCAA: valine, leucine and isoleucine). CC was processed by addition 3% urea. The first stage of *in vitro* test was done with 4 levels of molases 0, 5, 10 and 15% of dry matter (DM) of CC. The experiment was carried out using complete randomized design with 4 treatments and 4 replications. The second stage was also *in vitro* study of supplementation of two levels each for valine = V, leucine = L and isoleucine = I namely 0.1 and 0.2% of DM of ammoniated CC. There were 8 treatments combination of the BCAA as follow: A = V0.1 L0.1 I0.1; E = V0.2 L0.1 I0.1; B = V0.1 L0.1 I0.2; F = V0.2 L0.1 I0.2; C = V0.1 L0.2 I0.1; G = V0.2 L0.2 I0.1; D = V0.1 L0.2 I0.2; H = V0.2 L0.2 I0.2 and 1 control (V0.0 L0.0 I0.0). The experiments were done using completely randomized design with 9 treatments and 3 replications. The results showed that treated CC with urea was able to increase protein content by 78% (increased from 3.34% to 5.95%) while neutral detergent fiber (NDF) decreased by 15.4%, acid detergent fiber (ADF) by 7.9% and lignin 16.7%. Addition of molasses in ammoniated CC increased DM digestibility (P <0.05) by 7.5% (41.9 *vs* 43.51-46.26%) and NDF by 17.7% (38.41 *vs* 43.76 – 46.31%). Supplementation of BCAA resulted in the highest DM, OM and NDF digestibility (P <0.05) in the treatment of A, C, D and G. Compare to treament I, the digestibility of DM, OM and NDF in G treatment increased by 31.4%, 27.5% and 36.5%, respectively and produced the highest total population of rumen bacterial of 12.4 x 10^9 colonies /ml. It can be concluded that the digestibility of ammoniated CC increased by the supplementation of 5% molases and BCAA combination consisted of 0.2% valine, 0.2% leucine and 0.1% isoleucine.

Key Words: Corn Cob, Ammoniation, Molasses, Branched-Chain Amino Acid

INTRODUCTION

National production of corn grain in 2017 was 27.957 million tons (Pusdatin 2017). The potential of corn cob availability calculated based on ratio of grain, straw, husk and corn cob, was 55.0, 20.3 12.3 and 12.3%, respectively (Yulistiani et al. 2012). Corn cob production in 2017 was 6.251 million tons. The utilization of corn cob as feed was still limited. Some of dry corn cob was used as fuel, while most leaves were piled up and rotten in the field. Corn cob had low nutrient quality due to low protein (1.5-3.5%), high fiber (35-45%) high lignin (5.2%), and high cellulose (30%) contents (Yulistiani et al. 2012; Hastuti et al. 2011). Lignin in corn cob caused low nutrient availability and low dry matter digestibility (45.5%) and organic matter digestibility (42.5%) (Yulistiani et al. 2012). Lower digestibility (29.34%) was also reported by Setyadi et al (2013).

To increase the nutrient quality of fibrous feed, many processing methods have been done such as physical, chemical and biological or combination of these methods. Those treatments were reported able to increase the utilization and digestibility of agricultural by-products feedstuff (Van Soest 2006; Zain et al. 2010; Nurhaita 2010).

Physical treatment by grinding resulted in easier feed handling and for consumption, however it did not significantly increase feed digestibility. Increasing quality of roughage was reported by using chemical treatment of sodium hyadroxide (Adeniji 2010) or urea (Oji et al. 2007; Dean et al. 2008; Yalchi et al. 2009; Puastuti et al. 2010; Yulistiani et al. 2012, Ubwa et al. 2014). Urea treatment on rice straw was able to increase nutrient content increasing animal growth through improving rice straw palatability (Huyen et al. 2012), increased nutrient digestibility in dairy cattle (Wanapat et al. 2009) and increased NDF and ADF digestibility in goat and bull (Gunun et al. 2013). The increased of nutrient content and digestibility due to urea treatment was also reported in *Triticale* straw (Yalchi et al. 2009), cacao pod (Puastuti et al. 2010; Laconi & Jayanegara 2015), corn cob (Yulistiani et al. 2012), oil palm frond (Febrina 2012) and rice husk (Ubwa et al. (2014). The mechanism of urea treatment in improving nutrient content of low quality feed is through hemicellulose solubilization, swollen of plant cell wall and disruption of lignin and cellulose linkage (Yalchi et al. 2009). Consequently the digestibility of cellulose and hemicellulose was increased. This increased digestibility was not followed by the loss of lignin (Bata 2008). Differed to biological treatment, urea treatment in low quality feed is simple and applicative for small holder farmers.

The nutritive value of fibrous feed beside affected by processing method also affected by enzymatic digestion produced by rumen microbes. Various rumen microbes have important role in digesting feed in the rumen. Beside that rumen microbes also be used as protein source in ruminant. Rumen microbes at least supplied two third or 70-80% of amino acid required by ruminants (Chumpawadee et al. 2006; Pathak 2008). The higher rumen microbes population particularly cellulolytic bacteria produced more enzyme consequently increased fiber digestibility. Feeding low quality feeds with low protein and high fiber contents would limit its nutrient availability for microbial. (Puastuti 2009; Nurhaita & Ningrat 2011).

Fermentable energy source (sugar, starch) was needed for the growth of rumen microbials. Molases was fermentable source of carbohydrate and could be used as energy source for rumen microbes (Bata 2008). Amino acid was main nitrogen source for microbial synthesis in the rumen. Although cellulolytic rumen microbial was able to use ammonia, but amino acid and protein had better advantage compared to urea for microbial synthesis (McAllen & Smith 1983; Clark et al. 1992). High degradable protein will be fast degraded by rumen microbes in the rumen into amino acid and partly will be further degraded and produced ammonia. This ammonia is used as nitrogen source and as carbon skeleton source for rumen microbial synthesis. Therefore amino acid composition of feed protein is important for ruminant animals. Methionin, lysine, fenylalanin and threonine were amino acid needed by ruminants (Scholljegerdes et al. 2005). Other amino acid also needed by ruminants were valin, isoleusin, dan leusin (Volden 1999; Zain 2007). These three amino acids were known as *branched chain amino acid* (BCAA) in the rumen which would be decarboxylated and oxidative deaminated into branched-chain volatile fatty acid (BCVFA) which had important role in the activity of cellulolytic bacteria (Tedeschi et al. 2015). BCVFA could increase dry matter digestibility, increase rumen microbial growth and increase microbial function and enzyme activity in the rumen of sheep (Moharrery 2004). Considering results from the previous studies, this study was aimed to increase ammoniated corn cob digestibility through supplementation of molasses and combination of branch-chain amino acid valin, leusin, and isoleusin.

MATERIALS AND METHODS

Corn cob was obtained from Majalengka District, West Java Province. The cob was ground, then processed using ammoniation by urea addition at 3% of cob dry matter (DM) (Yulistiani et al. 2012). Corn cob and urea mixture was then kept anaerobically condition for 21 days. Ammoniated corn cob then dried and ground for chemical analysis and evaluated for its *in vitro* digestibility. First *in vitro* digestibility study was conducted to evaluate the requirement of energy source on the utilization of ammoniated corn cob as fiber source. Molasses supplementation as energy source was allocated in 4 levels namely 0, 5, 10, dan 15% of corn cob DM. The study was conducted through randomized complete design with four replications. Substance samples were incubated for 72 hour, which was previously prepared according to the method of Menke and Steingass (1988) and digestibility was measured according to the methods of Blummel et al (1997). Rumen fluid for *in vitro* study was taken from ram fed on fresh chopped elephant grass, supplemented with concentrate feed containing crude protein 16%. Parameter recorded were digentibility of DM, organic matter (OM) and neutral detergent fiber (NDF). The best molasses level in this in vitro digestibility was used in the second experiment in which the ammoniated corn cob and molasses mixture was supplemented by *branch-chain amino acid* (BCAA).

The second *in vitro* evaluation was carried out to study the effect of BCAA supplementation. BCAA supplemented were valin (V), leusin (L) and isoleusin (I) (L-Valine product by Himedia Laboratory Pvt., Ltd., and L-Leucine by Appli Chem). Levels of BCAA supplementation were 0.1 dan 0.2% of DM ammoniated CC. There were 8 combinations of the BCAA of treatment, which were: A (V0.1 L0.1 I0.1); B (V0.1 L0.1 I0.2); C (V0.1 L0.2 I0.1); D (V0.1 L0.2 I0.2); E (V0.2 L0.1 I0.1); F (V0.2 L0.1 I0.2); G (V0.2 L0.2 I0.1); H (V0.2 L0.2 I0.2) and I (V0.0 L0.0 I0.0). The study was conducted in randomized complete design with 9 treatments and 3 replications. The method used *in vitro* evaluation was similar to the first experiment. Parameter recorded were digestibility of DM, OM, and NDF, and number of population bacteria and protozoa.

DM, OM, crude protein (CP) and gross energy (GE) content of the samples were analysed using method of AOAC (1990). Whereas NDF, acid detergent fiber (ADF) and lignin were analyzed according to the methods of Van Soest et al. (1991). Bacterial and protozoal population were determined using *total plate count* method (Ogimoto & Imai 1981). Data obtained were analysed using analysis of variance. The difference between treatment mean were analysed using Duncan multiple range test. Statistical analysis was done using *soft ware* of SAS 9.0 (SAS 2002).

RESULTS AND DISCUSSION

Ammoniated corn cob

Physical condition of ammoniated corn cob was changed, after treatment and its texture was softer and had brownish color. The chemical analysis of the cob showed that ammonization increased the CP content by 78% (from 3.34 in untreated into 5.95% in urea treated corn cob). Chemical composition of untreated and urea treated corn cob are presented in Table 1. The increased of CP content in ammoniated corn cob was due to nitrogen fixation from urea catabolism by urease bacterial (Dean et al. 2008). The increase of CP content in this study was in agreement with the study reported by Oji et al. (2007). They reported that processing of corn by-products with 3% urea was able to increased CP content by 77%. Yadete (2014) reported that processing of wheat straw using 4% urea was able to increased CP content by 87.5% (from 3.2% into 6.0%).

The increase of CP content in rice husk due to urea treatment was also reported by Ubwa et al. (2014). Yulistiani et al. (2012) reported higher increased of CP content in corn cob treated with urea 3%, increasing the CP content up to 284.5%. While Ramirez et al. (2007) reported the CP content was increased by 125% and 270% in maize by-products with urea treatment levels of 4.5 and 6.5% respectively. The different in increase of CP content due to urea treatment in corn cob might be caused by the different level of urea used for treatment. Optimum level of urea in the process of ammonization was reported at the level of 3-5% (Gunun et al. 2013; Khejornsart & Wanapat 2010). Urea levels less than 3% only had function as preservative and it would loss as ammonia when higher than 5% (Khejornsart & Wanapat 2010). The length of storage (21 days) might vary the difference in CP content as the urea added was fractionating into ammonia and some evaporate during drying, so that the amount of N calculated as CP would be lower.

NDF content of ammoniated corn cob decreased by 15.5%, while ADF by 7.9% and lignin by 16.7%, whereas GE content decreased only by 2.5%. The results of the present study was in agreement with Nurfeta et al. (2008) who reported that urea

Table 1. Nutrient composition of corn cob untreated or ammoniated corn cob

Feed	DM	Protein	Ash	NDF	ADF	Lignin	GE
	---------------------------------- % -----------------------------						Kcal/kg
CC untreated	93.43	3.34	4.29	86.77	46.41	12.40	4111
CC amoniated	95.49	5.95	2.74	73.36	42.76	10.13	4214

DM = Dry matter; NDF = Neutral detergent fiber; ADF= Acid detergent fiber; GE= Gross energy treatment in wheat straw reduced NDF content. Chemical treatment by urea addition cause disruption the linkage between cell wall component particularly linkage between lignin and cellulose or hemicellulose so that digestibility by enzymatic become easier. Previously Sumarsih et al. (2007); Mapato et al. (2010) stated that ammonia treatment increased digestibility by lossen lignocellulose bound consequently carbohydrate digestibility increased. Beside that urea treatment also increased feed palatability. The increase of digestibility in urea treated in rice straw caused the increase in feed consumption (Hailu et al. 2011)

Effect of molasses supplementation on digestibility of ammoniated corn cob

Different levels of molasses supplementation significantly (P<0.05) affected DM, OM and NDF digestibility. Nutrient digestibility of ammoniated corn cob supplemented by molasses is presented in Table 2.

Digestibility evaluation of DM, OM and NDF of ammoniated CC with molasses addition as energy source, was significantly different (P<0.05) among levels of molasses supplementation. Digestibility value of ammoniated CC supplemented by molasses presented in Table 2.

Result from Duncan analysis showed that molassess supplementation up to 5% was not able to increased DM and OM digestibility. The digestibility was increased (P<0.05) at 10 and 15% molasses supplementation compare to control (without molasses supplementation). The NDF digestibility was increased (P<0.05) at 5% molasses supplementation compared to control, but this was not significantly different from 10 and 15% supplementation. DM digestibility of 41.9% of ammoniated CC in this study was similar to the results reported by Prastyawan et al. (2012). Who reported that fermented ammoniated corn cob without starter microbes for 4 weeks incubation resulted in DM digestibility of 41.3%. The value was higher than reported by Setyadi et al. (2013) which was 29.34%.

Compared to control, the average incrase in DM digestibility due to molasses supplementation was 9.2% (41.9 *vs* 43.51-46.26%), whereas the incrase in NDF digestibility was 17.7% (38.41 *vs* 43.76-46.31%) (Table 2). The increase digestiility with the increase of urea treatment could be due to urea treatment producing nitrogen in the form of NH3. Similar results was also reported on nitrogen supplementation in rice straw which was able to improve deficiency of CP content, consequently the straw digestibility was increased due to the ammonia (NH3) availability which was needed for the activity of rumen microbial for the fermentation of feed (Wanapat et al. 2009). Microbial protein synthesis required NPN and carbohydrate source from feed (Al Qori'ah et al. 2016). Therefore, the high potential of ammonia had to be synchronized with availability of fermentable energy. Ammoniated feed which directly fed to ruminants will result in imbalanced N caused by high N degradation but lack of fermentable carbohydrate and consequently more N release which in turn caused low feed digestibility. Therefore this feed should be balanced with fermentable carbohydrate which will optimize rumen microbial activity. The available fermentable carbohydrate ammonia will be converted into protein microbial and stimulate multiplication of rumen microbial, which will be able to increase feed digestibility (Bata 2008).

The increase of rumen microbial population which produces more enzymes resulted in the increased feed digestibility. Therefore in the present study ammoniated corn cob as feed supplemented with fermentable carbohydrate such as molasses which was expected to be able to optimize the activity of rumen microbial through the available N in the form of ammonia and energy which needed for rumen microbial multiplication and optimizing microbial activity in

Table 2. Effect of molasses supplementation on nutrient digestibility of ammoniated corn cob

Molasses levels	Digestibility		
	Dry Matter	Organic Matter	Neutral Detergent Fiber
		------------------------%--------------------	
0%	41.90±1.02^{b}	43.19±0.95^{b}	38.41±1.07^{b}
5%	43.51±0.77^{ab}	44.84±0.94^{ab}	43.76±1.50^{a}
10%	45.30±2.00^{a}	46.56±1.98^{a}	45.53±1.63^{a}
15%	46.26±2.57^{a}	47.35±2.60^{a}	46.31±3.35^{a}

Values in same column with different superscripts indicates significantly different (P<0.05) supplementation for ruminants fed on fibrous feed with carbohydrate fermentation due to the availability of ammonia and energy for the growth of rumen microbial. Crude protein degradation in ammoniated corn cob produced intermediate products in the form of ammonia. With the availability molasses as energy source, rumen microbes will incorporate with NH3 as source of N for protein synthesis of the microbes, particularly cellulolytic bacteria which in turn this bacteria will digest fiber. Some of rumen microbial required N for microbial synthesis in the form of ammonia. Minimum concentration required was about 3.57 mM (Satter & Slyter 1974) or in the ranges of 3.57-7.14 mM that had to be produced from protein in the diet (Swandyastuti and Rimbawanto, 2015). The increase of molasses levels resulted in non-significant increase of NDF digestibility, this could be caused by excessive energy available wich was not balanced with available N. The simultaneous available energy and nitrogen was essential to be to provide nutrient needed by rumen microbes.

In the present study, though digestibility of ammoniated CC supplemented by molasses increased in DM digestibility, but somehow still lower than of elephant grass digestibility (60.9%) (Santoso et al. 2009). Therefore, the process of treating crn cob with urea, it had to be combined with fermentable carbohydrate supplementation to increase digestibility due to the increase rumen microbial population. Principally fiber digestibility has to be close relation to enzyme produced by rumen microbial. Basic nutrients needed for rumen microbial synthesis was available energy balanced with nitrogen in the form of ammonia. In the present study, molasses containing high glucose was added. As stated by Nurhaita et al (2014) that nutrient required for microbial protein synthesis was energy, nitrogen, mineral and amino acid in the form of BCAA. Therefore it is proper in order to increase digestibility of ammoniated CC can be done by supplementation of branched-chain amino acid (BCAA) to stimulate the growth of rumen microbial.

Effect of BCAA supplementation on ammoniated CC

Digestibility value of ammoniated CC with supplementation of 5% molasses and BCAA is presented in Table 3. Ammoniated CC feed as fiber source with 5% molasses increased NDF digestibility by 13.9% (Table 2). Ammoniated CC digestibility could be increased by supplementation of branch-chain amino acid through stimulation of protein microbial synthesis. Digestibility value of ammoniated CC is presented in Table 3. The digestibility of DM, OM and NDF of corn cob was increased ($P<0.05$) by BCAA supplementation. The significant increase of low quality, resulted in better rumen environment for digestibility due to BCAA supplementation was in treatment A, C, D and G. The digestibility of DM, OM and NDF in ammoniated CC (Tabel 2) was improved by 35.03%, 33.38% and 25.42%, respectively.

The results of present study supported previous research reported by Zain et al. (2008) that BCAA (valin 0,1%, leusin 0,15%, isoleusin 0,2%) supplementation in palm press fiber basal ration was able to increased DM and OM digestibility by 22,84% (from 56,47 to 69,37%) and 21,61% (from 57,84% to 70,34%), respectively. Although DM, OM and NDF in treatment G was not statistically significantly differ from treatment A, C and D, treatment G was better in term of increase total bacterial population (12,4 x 10^9 coloni/ml) (Table 4).

In the present study supplementation of BCAA might be used as precursor for microbial protein synthesis which was able to increase bacterial population thereby increased fiber digestibility of ammoniated CC.

Bacteria population increased more than 1.5 times compared to control. In contrast, protozoa population (3.50 x 105 cell/ml) in BCAA supplementation was significantly lower compared to control (13.5 x 105 cell/ml). This results indicated that combination of BCAA supplementation in treatment G was able to support the growth of rumen bacterial.

Branched-chain amino acid had significant role in increasing protein synthesis in human and animal (Zhang et al. 2017). Amino acid in the rumen is utilized by rumen microbes to synthesize protein microbial. Rumen bacteria, particularly cellulolytic bacteria required branched-chain fatty acid (BCFA) consisted of isobutyric, 2 metil butiryc, and valeryc acid as source bacterial carbon skeleton. This BCFA produced from decarbocilated and deaminated BCAA. Most BCAA in the rumen produced from fermentation of protein in the diet and lysis of rumen microbial (Zhang et al. 2017). With the low quality of agroindustrial by-products in term of low content in BCAA, therefore BCAA (valin, isoleucin, and leucin) supplementation in agroindustrial by-producs basal diet is needed to increase rumen microbial population and fiber digestibility.

Based on treatment of BCAA supplementation in ammoniated CC, there was indication of negative correlation between bacterial and protozoal population in the rumen (Figure 2). This correlation follows the equation $y = -2.1338x + 27.99$; $R2 = 0.7756$.

Feeding diet containing high fiber such as corn cob with high bacterial population and low protozoal population (Treatment G, Table 3) will be very beneficial due to bacteria population. Fibrolytic bacteria particularly is needed to increase fiber digestibility this fiber digestibility produced energy in the form VFA which is used as energy source for ruminants.

Table 3. Effect of combination of BCAA valin, leusin and isoleusin supplementation on ammoniated corn cob (CC) digestibility

Treatments	Digestibility		
	DM	OM	NDF
	------------------------------ % -------------------------		
A	59.54±0.37^{a}	59.67±0.38^{a}	55.85±2.18^{a}
B	55.76±1.42^{ab}	57.28±1.48^{ab}	51.84±1.31^{ab}
C	58.88±1.48^{a}	60.24±1.54^{a}	55.19±1.37^{a}
D	58.51±2.3^{a}	59.84±2.41^{a}	54.30±2.12^{a}
E	52.85±0.60^{ab}	54.57±0.91^{ab}	49.07±0.91^{ab}
F	51.54±2.75^{ab}	53.72±2.86^{ab}	47.22±2.53^{ab}
G	58.06±2.19^{a}	59.49±2.35^{a}	54.19±1.99^{a}
H	51.10±3.51^{ab}	53.71±3.69^{ab}	45.33±3.22^{ab}
I	44.18±6.47^{b}	46.67±6.36^{b}	39.70±6.13^{b}

DM = Dry matter; OM = Organic matter; NDF = Neutral detergent fiber
A = (V 0.1, L 0.1 I 0.1); B = (V 0.1, L 0.1, I 0.2); C = (V 0.1, L 0.2); D = (V 0.1, L 0.2, I 0.2); I 0.1); E = (V 0.2, L 0.1, I 0.1)
F = (V 0.2, L 0.1, I 0.2); G = (V 0.2, L 0.2, I 0.1); H = (V 0.2, L 0.2, I 0.2); I (control)= (V 0.0, L 0.0, I 0.0).
Values in same column with different superscripts indicates significantly different ($P<0.05$).

Table 4. Effect of combination of valin, leusin and isoleusin supplementation in ammoniated CC on rumen microbial population

Treatments	Bacteria (10^9 col/ml)	Protozoa (10^6 cell/ml)
A	2.80±0.14^{f}	2.58±0.12^{a}
B	6.98±0.60^{c}	1.70±0.10^{c}
C	3.87±0.12^{e}	2.23±0.03^{b}
D	6.06±0.56^{d}	1.27±0.11^{d}
E	7.46±0.83^{c}	0.85±0.15^{e}
F	10.11±0.08^{b}	0.55±0.05^{f}
G	12.40±1.1^{a}	0.50±0.05^{g}
H	7.12±0.73^{dc}	1.20±0.05^{d}
I	4.63±0.38^{e}	1.35±0.05^{d}

DM = dry matter; OM = Organic matter; NDF = Neutral detergent fiber,
A = (V 0.1, L 0.1 I 0.1); B = (V 0.1, L 0.1, I 0.2); C = (V 0.1, L 0.2); D = (V 0.1, L 0.2, I 0.2); I 0.1); E = (V 0.2, L 0.1, I 0.1)
F = (V 0.2, L 0.1, I 0.2); G = (V 0.2, L 0.2, I 0.1); H = (V 0.2, L 0.2, I 0.2) I (control)= (V 0.0, L 0.0, I 0.0).
Values in same column with different superscripts indicates significantly different ($P<0.05$)
.

Nutrition needed by protozoa is protein and fermentable energy in the form of starch or sugar, on the other hand corn cob contain carbohydrate mostly in the form of fiber this condition retarded the growth of protozoa. Mustofa et al. (2012) reported that fermentation of ammoniated CC which was processed using commercial starter produced 113 mM VFA, increased cellulolytic microbial population increased VFA production as energy source for livestock. The amount of supply protein from microbial biomass into small intestine could be up to 50-80% from total absorbed protein (Bach et al. 2005). Treatment G (V 0.2, L 0.2, I 0.1) produced highest bacterial population among four BCAA combination. Fibrous fermented bacterial such as *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Butyrivibio fibrisolvents* required branched-chain fatty acid (BCFA) such as isobutyric, isovaleric, valeric, and 2-methylbutyric acids for their growth (Zhang et al. 2013). BCFA could be obtained from feed protein or recycled of rumen bacterial protein through oxidative deamination and decarboxylation from valin, leucin, and isoleucin (Moharrery 2004). The best BCAA combination in the present study was different to the study reported by Zain et al. (2008).

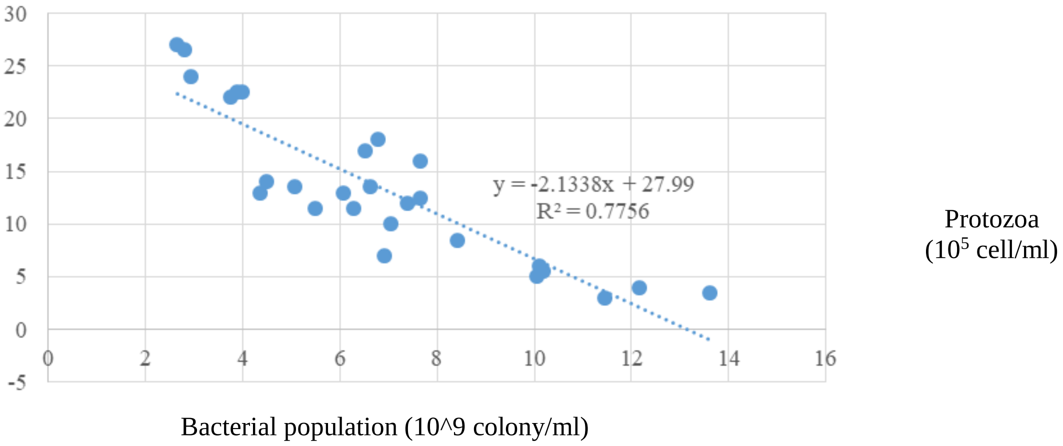

Figure 2. Corellation between bacteria and protozoa population in the *in vitro* rumen of amoniated CC supplemented with BCAA

They reported higher rumen bacterial population in sheep fed on palm press fiber complete diet supplemendt with valin 0.1%, leucin 0.15% dan isoleucin 0.2% than control diet (18.88 x 10^{10} col/ml *vs* 10.9 x 10^{10} col/ml). The different results in the present study with Zain et al. (2008) study in term of bacterial population was due to the difference quality nutrient supply to support bacterial synthesis.

The used of BCAA in the field particularly for small holder farmers is quite expensive, this can be as constraint in the used of BCAA. Therefore it is needed to search alternative for BCAA sources which is cheap and easily obtained. Some of forage protein such as leucaena, gliricidia, calliandra and cassava leaves contain high BCAA. According to Hartadi et al. (1997) leucaena contained valin 1.51%, leucin 2.33% and isoleucin 1.3%. Widiawati et al. (2007) reported that leucaena contained valin 637 mg/g N, leucin 504 mg/g N, isoleucin 536 mg/g N, *Gliricidia maculate* leaves contained valin 646 mg/g N, leucin 521 mg/g N, isoleucin 563 mg/g N. Caliandra leaves contained valin 685 mg/g N, leucin 543 mg/g N, isoleucin 564 mg/g N. Cassava leaves contained valin 1%, leucin 1.3% and isoleucin 0.84% (Puastuti 2013, Unpublished). Therefore in field application utilization of BCAA can be implemented through forage protein source supplementation which is rich in amino acid with BCAA composition closed to treatment G (valin 0.2%, leusin 0.2% dan isoleusin 0.1%). However, attention need to be given type of legume forage source which will affect the composition of total ration and may have different effect, therefore further research on the use of forage as BCAA source need to be done.

CONCLUSION

Processing corn cob using urea treatment at 3% DM of corn cob was able to increase CP content by 78%. Supplemented ammoniated CC with molasses 5% and BCAA combination with valin 0.2%, leusin 0.2% dan isoleusin 0.1% resulted in highest DM, OM and NDF diegestibility of 58.06%, 59.49%, 54.19% respectively as indicated by the highest bacterial population and the lowest protozoa population. The utilization of ammoniated CC is suggested supplemented by molases 5% and BCAA combination valin 0.2%, leusin 0.2% dan isoleusin 0.1%.

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Growth and Feed Utilization of Boer x Kacang Crossbred Goats Offered Total Mixed Rations of Different Protein and Energy Levels

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ABSTRAK

Ginting SP, Simanihuruk K, Antonius, Tarigan A. 2017. Pertumbuhan dan pemanfaatan pakan kambing persilangan Boer x Kacang yang diberi pakan komplit dengan kandungan protein dan energi berbeda. JITV 22(4): 188-195. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1782

Penelitian bertujuan untuk mengevaluasi pertumbuhan dan pemanfaatan pakan pada kambing persilangan Boer x Kacang yang diberi pakan komplit dengan taraf protein dan energi berbeda. Empat kombinasi pakan komplit yang mengandung 16 atau 18% protein kasar dan 2650 atau 2850 Kkal ME/kg BK digunakan dalam penelitian acak lengkap dengan pola faktorial. Sebanyak 28 ekor kambing persilangan Boer x Kacang (14,5±1,14 kg) dan umur antara 4-5 bulan secara acak dialokasikan kepada salah satu dari keempat perlakuan pakan komplit (7 ekor ternak/TMR). Pengaruh perlakuan pakan dianalisis dengan analisis sidik ragam model linier dan apabila terdapat pengaruh perlakuan selanjutnya di uji dengan uji *Duncan's Multiple Range*. Terjadi peningkatan konsumsi BK (P<0,05) jika kandungan *energy metabolism* pakan meningkat dari 2650 menjadi 2850 Kkal/kg BK, namun konsumsi BK tidak dipengaruhi (P>0,05) oleh taraf protein kasar dalam pakan. PBBH tidak meningkat (P>0,05) dengan peningkatan kandungan protein kasar dan *energy metabolism* dalam pakan. Disimpulkan bahwa dengan konsumsi pakan komplit pada kambing persilangan Boer x Kacang yang tergolong tinggi, maka kandungan protein kasar 16% dan densitas *energy metabolism* 2850 Kkal/kg BK dapat menghasilkan PBBH yang tinggi.

Kata Kunci: Kambing, Pakan Komplit, Taraf Protein, Taraf Energi

ABSTRACT

Ginting SP, Simanihuruk K, Antonius, Tarigan A. 2017. Growth and feed utilization of Boer x Kacang crossbred goats offered total mixed rations of different protein and energy levels. JITV 22(4): 188-195. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1782

The aim of this study was to evaluate the growth of and feed utilization by Boer x Kacang crosses goats fed on total mixed ration differing in protein and energy levels. Four total mixed rations combination were formulated to contain 16 and 18% crude protein and 2650 and 2850 Kcal ME/ kg DM (dried matter) energy density. Twenty-eight male Boer x Kacang crosses goats (14.5 ± 1.14 kg) and of age ranging from 4 to 5 months were randomly allocated to one of these four TMRs (total mixed rations) (7 animals/TMR). The effects of dietary treatment were assessed using the general linear model and significance of the diet effects was detected using Duncan's multiple range test. Dry matter intake increased as metabolizable energy density of diet increased from 2650 to 2850 Kcal/kg DM, but it is not affected by increasing crude protein level from 16 to 18%. The average daily gains were not improved (P>0.05) as the crude protein levels and metabolizable energy density of diet increased. Crude protein levels and ME density did not affect (P>0.05) the DM, OM and energy digestibility, but NDF digestibility was affected by the ME density of diets (P<0.05). Daily N intakes were greater (P<0.0%) in goats received diets higher in the crude protein and metabolizable energy levels. At this high rate of feed intake this type of goats are able to gain optimally when offered feed with crude protein level of 16% and metabolizable energy density of 2850 Kcal/kg DM.

Key Words: Goats, Total Mixed Ration, Protein Level, Energy Level

INTRODUCTION

Crossbreeding schemes between introduced Boer goats and indigenous Kacang Goats have been undertaken at the Goat Research Institute at Sungai Putih, North Sumatera. The production performances evaluation of the Boer x Kacang crossbreds are currently in progress, and the ultimate goal of this program is to create a 50% Boer and 50% Kacang genotype with superior performance potentials compared to that of native Kacang goats, particularly in the trait of growth rate and body weight. It is generally accepted that crossbred genotypes would only express their performances to their genetic potentials when raised under optimum production system (Bryant et al. 2005), in which nutrition plays a critical element. Improving the nutrition status of ruminants through increasing the intake of main nutrients such as crude protein and metabolizable energy resulted in increased dry matter intake and digestibility, N retention and liveweight gain (Rios-Rincon et al. 2014; Singh et al. 2013). However, the magnitude of the responses vary greatly across breeds (Negesse et al. 2001; Hwangbo et al. 2009). This is indicative of different protein and energy requirements for maintenance and production of various genotype of goats under different production system.

In Indonesia, the integration of ruminant animals including goats with palm oil plantation is being promoted as one of the prospective production systems. The availability and efficient utilization of various types of feeds to support animal production under this production system play a vital role. The aim of this study was to evaluate the growth and feed utilization of Boer x Kacang crossbreds offered diets differing in the crude protein and metabolizable energy levels in the form of totally mixed ration and using palm oil fronds as the sole roughage.

MATERIALS AND METHODS

Diets, animals and experimental design

Four total mixed rations (TMR) using palm oil fronds as the sole roughage were formulated to have different crude protein levels (16% and 18%) and metabolizable energy levels (2650 Kcal/kg DM and 2850 Kcal/kg DM). Ingredients and the calculated chemical compositions (DM basis) of the four TMRs are presented in Table 1. Oil palm fronds were obtained from the oil palm plantations. After removing the leaves, the stems were ground using shredder and mixed thoroughly with other ingredients. Twenty-eight male Boer x Kacang crossed goats of similar body weight (14.5±1.14 kg) and age (4 to 5 months old) were randomly allocated to one of the four dietary treatments each replicated seven times with one goat per replicate. Animals were put in individual metabolic cage equipped with troughs and water container. Feeds were offered ad libitum by allowing refusal at approximately 10%. The animals were fed twice daily at 08:00 and 14:00 each of equal amounts. Water was available at all the times. Animals were adapted to their pens and dietary treatments for 14 days and the feeding trial lasted for 90 days. During the feeding trial feeds offered and refusals were recorded daily while animal's body weights were measured biweekly. Following the feeding trial a digestion trial was performed during which offered feed and individual refusals, feces and urine were collected and sampled daily for five consecutive days. Faecal samples (10%) were collected and pooled by animal. Parts of the faecal samples were processed for DM determination (80°C for 48 h). Other parts were dried at 600°C, ground to pass through 1 mm screen and stored in refrigerator for laboratory analyses. Urine from individual animal was collected in plastic container containing 100 ml of 10% sulphuric acid to maintain pH below 3.0 and keep in a freezer until analysed. Blood was sampled at the end of the digestion trial from the jugular vein just before morning meal. Blood samples were stored at -20°C before analyses.

Laboratory analyses

Feeds, individual refusals, faeces were analyzed for dry matter (DM), organic matter (OM) and nitrogen according to the standard procedures (AOAC 2012) and for neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the procedures described by Van Soest et al. (1991). Urine was analyzed for nitrogen. Blood samples were analyzed for glucose and urea-N using Vitalab Flexor auto analyzer kits.

Statistical analyses

The experiment was arranged in a completely randomized design with a 2 x 2 factorial arrangement. Each treatment consisted of seven replicates. The effects of dietary treatments on daily gains, intake, digestibility, nitrogen balances, blood metabolites were

Table 1. Ingredient and chemical compositions of the experimental diets

ME (Kcal/kg)	2650 Kcal/kg DM		2850 Kcal/kg DM	
CP (%)	16% CP	18% CP	16% CP	18% CP
Ingredients (%)				
Indigofera leaf meal	20.0	20.0	20.0	20.0
Palm kernel cake	29.0	19.0	10.0	8.0
Molasses	5.0	5.0	5.0	5.0
Soybean meal	14.0	24.0	18.0	27.0
Bone meal	1.0	1.0	1.0	1.0
Mineral premix	1.0	1.0	1.0	1.0
Corn	0	0	19.0	10.0
Palm oil fronds	30.0	30.0	30.0	30.0
Chemical compositions				
CP,%	15.95	17.97	15.81	18.10
Ca,%[a]	0.53	0.59	0.55	0.62
P, %[b]	0.32	0.31	0.30	0.33
NDF,%	43.57	46.46	45.12	44.71
ADF,%	31.33	32.81	32.39	31.53
DE (Kkal/kg DM)	2680	2660	2872	2865

[a,b]Calculated

assessed statistically using analysis of variance and a general linear model (GLM) procedure in SAS (1989). The statistical model used is $Yijk = \mu + \square i + \beta j + \square\beta ij + \varepsilon ijk$ where Yijk is the individual observations, μ is the overall mean, $\square i$ (i=1,2) is the energy levels, βj (j=1,2) is the protein levels and $\square\beta ij$ is the energy-protein interactions and εijk is the unexplained variation assumed normally and independently distributed. The significance of the diet effects was determined at $P \leq 0.05$. The treatment means were compared using the Duncan's multiple range test**.**

RESULTS AND DISCUSSION

Feed intake and daily gains

The effects of dietary treatments on the feed intake and growth performances are shown in Table 2. There was no interaction between energy and protein levels on feed intake. The DM intake was affected by energy level but not by the protein levels of the diets. Similar trend was seen when DMI was expressed as percentage of body weight. Goats fed on high energy level had higher DM intake. The level of DM intake in all dietary treatments was considerably high, ranging from 5.1 to 5.7% BW. These levels of dry matter intake are comparable to the observation of Limea et al. (2009) in Carribran goats that reached level of feed intakes of 5.1 to 5.4 % BW. This high feed intake level in the present experiment could be associated with the type of diets offered as totally mixed ration in which all feed ingredients have been ground. Thus, the physical fill effect of the diet which could be a limiting factor of feed intake in ruminant animals may have been minimal in the present experiment. As goat kids fed on the higher energy diet the feed intake was higher as well. This might indicate that the high energy level used in this study may have not reached the dietary energy density that will limited DM intake. Rios-Rincon et al. (2014) found that the DM intake was limit when the level of metabolizable energy of diet is 3050 Kcal/kg. At lower metabolizable energy diets (ranging from 2380 to 2720 Kcal/kg), Wang et al. (2015), however, did not detect the effect of dietary energy level on feed intake in Hanian black goats. The absence of crude protein level effects on feed intake observed in this study is in agreement with Wang et al. (2015) that offered goats with diets CP levels of 15 and 17% or with Rios-Rincon et al. (2014) offered lambs with diets of 14.5 and 17.5% CP contents. Some studies (Ebrahimi et al. 2007; Kioumarsi et al. 2008; Wang et al. 2015) indicated that the effects of crude protein levels of diets on feed intake to be more significant when the protein levels were set below 14%, although Boonsaen et al. (2017) did not find the effect of protein levels on feed intake in steers fed diets with CP level of 12 and 14%.

Table 2. Dry matter intake and daily gains of Boer x Kacang crossbred kids offered total mixed ration with difference in energy and protein levels

Item	Energy level (Kcal ME/kg)				Main effect			
	2650		2850		Energ level (Kcal ME/kg)		Protein level (%)	
	16 % CP	18 % CP	16 % CP	18 % CP	2650	2850	16	18
DMI, g/d	957.8±46.27^a	1006.5±35.16^a	1113.3±43.78^b	1125.6±38.92^b	982.15^a	1119.45^b	1035.55	1059.90
DMI, g/kg BW	51.15±4.75^a	52.2±3.67^a	56.54±4.59^b	57.11±3.44^b	51.68^a	56.83^b	53.85	54.66
Initial BW, kg	14.5±0.93	13.9±0.97	15.31±1.41	14.97±1.58	14.2	15.14	14.91	14.44
Final BW, kg	22.95±0.71	23.73±1.15	25.57±0.43	24.44±1.69	23.34	25.01	24.26	24.09
ADG, g	97.21±6.17	106.24±5.15	112.15±4.96	108.87±7.88	101.73	110.51	104.68	107.56

^{a,b} Different superscripts in the same column significantly difference ($P<0.05$); ns: non significantly difference

Table 3. Apparent digestibility coefficients (%) of nutrients in goats kids fed diet containing varying energy and protein levels

Item	Energy level (Kcal ME/kg)				Main effect			
	2650		2850		Energ level (Kcal ME/kg)		Protein level (%)	
	16 % CP	18 % CP	16 % CP	18 % CP	2650	2850	16	18
Dry Matter	71.98±4.96	68.44±3.83	70.11±4.33	67.60±3.98	70.21	68.86	71.05	68.02
Organic Matter	75.50±3.52	71.14±4.89	73.43±2.31	69.51±4.26	72.82	71.47	73.96	70.33
NDF	59.81±4.58 ^{a}	57.56±5.85^a	53.05±3.98^b	54.26±2.39b^b	58.69^a	53.66^b	56.43	55.91
Energy	76.23±2.9	74.35±4.72	75.99±5.21	71.75±3.89	75.29	73.87	76.11	73.05

^{a,b} Different superscripts in the same column significantly difference ($P<0.05$); ns: non significantly difference

Protein and energy level of diets did not significantly (P>0.05) affect average daily gains and final weights of the goats kids. As expected, increasing in feed intake resulted in increased crude protein and metabolizable energy intake. The calculated daily crude protein intake of goats in the low and high protein diets were 166 and 191g, respectively, while the metabolizable energy intake was calculated to be 2623 and 3108 Kcal/day in low and high energy diets, respectively. These figures are greater than those recommended by Mandal (2005) as daily crude protein requirement (84g) and metabolizable requirement (1800 Kcal/d) for growth of the Indian local goats. The crude protein and metabolizable energy requirement of Boer x Kacang crossbred goats used in this study might be higher than those of Indian goats, but the level of CP protein and the metabolizable energy intake in all dietary treatments may have been sufficient to fulfill the requirement for their optimal growth as indicated by the relatively high growth rate of goats in all dietary treatments. A greater growth rate was reported by Ryan et al. (2007) in Boer crossbred goat (125 g/d) and by Mahgoub et al. (2005) in Omani goats (133 g/d) when the animals were fed on high-concentrate diets.

Apparent nutrient and energy digestibility of dietary treatments are shown in Table 3. The results indicated that there was no significant effect (P>0.05) of different metabolizable energy and crude protein levels on the apparent DM and OM and energy digestibility except the NDF digestibility, and no protein and energy level interaction was discovered. The apparent digestion coefficient of DM, OM and energy in all dietary treatments were considered to be high, which might be related to the relatively great proportion of highly digested ingredients used in the diets. Also, the physical process of diet in which each ingredient was ground to form the total mixed ration might have contributed to this relatively high digestibility coefficient of the nutrients. These results are comparable to the findings of Robinson et al. (2006) or Elamin et al. (2012) working on Nubian and dessert goats. Karimizadeh et al. (2017) discovered that processing feed into complete feed block or mash can improve the DM digestibility. The NDF digestibility was greater (P<0.05) in goat kids offered diet with lower energy density. This might be related to the significantly lower intake level of goat fed diet with low energy density. Although feed retention time was not measured in this study, lower feed intake may resulte in longer retention time of the feed in the reticulo rumen which allowed the animal to better digest the feeds.

The N balance by goat kids under varying dietary energy and protein levels is presented in Table 4. There was no interaction (P>0.05) between energy levels and protein levels on the N balance. The daily average N intake by goat kids was significantly affected (P<0.05) by the energy and crude protein levels of the diet. Goats received diets with higher energy and protein levels consumed greater N compared to those fed diets of lower energy and protein levels. The fecal N output was not influenced by dietary energy and protein levels (P>0.05), although numerically, there was a trend that fecal N output was greater in goats when fed diets with higher protein and energy levels. A positive association between N intake and fecal N output has been observed by several authors (Pattanaik et al. 2003; Yang et al. 2014). Urinary N output was influenced (P<0.05) by the dietary protein levels, but not by dietary energy levels. The greater urinary N output found in goat offered diet with higher protein levels in the present study was in accordance with results reported by several authors (Lohakare et al. 2006; Cantalapiedra-Hijar 2009). A possible increase in the endogenous N losses due to increased feed intake in the higher protein level group could relate to the increasing urinary N output. The N retention (g/d) and N absorbed (as percentage of N retention) were affected (P<0.05) by the dietary protein level, but not by dietary energy levels. N retention is considered as the most common indicator of the protein nutrition status of ruminants. In the present study, positive N retention was observed in all dietary treatments and it is well accepted that N retention depends on the intake of nitrogen and the amount of fermentable carbohydrate of the diet. The greater N retention in the high protein level compared to the low protein level found in the present study might be associated the higher feed intake level. The similar N retention (as percentage of N intake) may be an indicative of the adequacy of metabolizable energy intake at the high or low protein level.

Blood metabolites are presented in Table 5. There was no interaction of energy x protein. Concentration of urea N was not affected by the dietary energy level but it was greater P<0.05) in goats offered diet with higher protein level. The source of plasma urea-N was reported to be from the NH3 liver conversion of excess amino acids and from ruminal degradation of dietary crude protein (Huntington et al. 2001). Thus, the higher BUN in the high dietary protein goats in present study should be from the greater crude protein intake that resulted in more amino acids transferred to the liver and more crude protein has been degraded in the rumen. The greater urinary N excretion in goats fed dietary with high protein level (Table 4) may be indicative of more protein has been degraded in the rumen, since there is a linear increase in urinary N with increasing protein degradation in the rumen (Javaid et al. 2008; Kalscheur et al. 2006). Plasma glucose concentration was higher (P<0.05) in goats fed high energy level, but it was not affected the dietary protein levels.

Table 4. Nitrogen balance of goat kids fed diets with different energy and protein levels

Item	Energy level (Kcal ME/kg)				Main effect			
	2650		2850		Energ level (Kcal ME/kg)		Protein level (%)	
	16 % CP	18 % CP	16 % CP	18 % CP	2650	2850	16	18
N intake (g/d)	23,44±1.02	26.94±1.26	28.16±1.64	32.59±1.37	25.19^{a}	30.38^{b}	25.80^{a}	29.77^{b}
Fecal N (g/h)	8.35± 0.343	8.84±0.22	9.58±0.39	10.56±0.42	8.60	10.07	8.97	9.70
Urine N (g/d)	4.66±0.33	6.96±0.41	5.02±0.30	6.50±0.37	5.81	5.76	4.84^{a}	7.73^{b}
N absorbed (g/d)	18.78±0.74	19.98±0.66	23.14±0.53	25.49±0.34	19.38	24.32	20.96	22.74
N Retention (g/d)	11.43±0.05	13.14±0.04	11.81±0.06	15.27±0.05	12.29	13.54	11.62^{a}	14.21^{b}
% intake	46.77±2.18	45.40±3.05	48.61±1.88	47.23±2.79	46.09	47.92	47.69	46.32

Table 5. Blood metabolites in goats fed two levels of dietary energy and protein

Item	Energy level (Kcal ME/kg)				Main effect			
	2650		2850		Energ level (Kcal ME/kg)		Protein level (%)	
	16 % CP	18 % CP	16 % CP	18 % CP	2650	2850	16	18
Urea-N (mg/dl)	24.08±1.76	29.01±1.64	26.33±1.72	33.06±2.62	26.55	29.70	25.21^{a}	31.04^{b}
Glucose (mg/dl)	66.29± 3.21	68.96±2.18	74.04±3.22	78.32±3.68	67.63^{a}	76.18^{b}	70.17	73.64

The plasma concentration ranged from 66 to 78 mg/dl and was comparable to those reported by Karimizadeh et al. (2017) fed goats diet with metabolizable energy concentration of 2500 Kcal/kg DM. This plasma glucose levels may indicative of adequate energy intake at both levels and related to the relatively high DM intake of the goats (Table 2). Sing et al. (2013) found lower plasma glucose level (47-52 mg/dl) at lower DM intake (500-600 g DM/day) compared to the DM intake in the present study. This is supported also by the insignificant effects of dietary energy levels on the blood N level, since energy deficiency could result in in reduced blood glucose with concomitant increase in blood urea (Yurtman et al. 2002). The insignificant effects of dietary protein level on the concentration of blood glucose observed in the present study were similar to result study of Meng et al. (2016) offered goats diet with crude protein level of 10, 12.5 and 15%.

CONCLUSION

The present study shows that the Boer x Kacang crossbred goats have high level of DM intake when offered feeds in the form of total mixed rations. At this high rate of feed intake this type of goats are able to gain optimally when offered feed with crude protein level of 16% and metabolizable energy density of 2850 Kcal/kg DM.

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Anticoccidial Activity of Artemisinin and Extract of *Artemesia annua* Leaves in Chicken Infected by *Eimeria tenella*

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ABSTRAK

Wiedosari E, Wardhana AH. 2017. Aktivitas antikoksidia artemisinin dan ekstrak daun *Artemesia annua* pada ayam yang diinfeksi dengan *Eimeria tenella*. JITV 22(4): 196-204. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1622

Penggunaan antikoksidia pada ayam yang terus menerus telah memicu timbulnya resistensi obat dan residu dalam jaringan, sehingga perlu dicari obat alternatif yang berbasis herbal. Tujuan penelitian ini adalah untuk menguji aktivitas antikoksidia artemisinin dan ekstrak daun *Artemesia annua* pada ayam yang diinfeksi dengan *Eimeria tenella*. Sebanyak 35 ayam jantan strain Cobb dibagi menjadi tujuh perlakuan dengan lima ulangan, yaitu kelompok ayam normal (P I), diinfeksi tanpa pengobatan (P II), diinfeksi dan diobati dengan 8,5 ppm, 17 ppm, 34 ppm masing-masing untuk P III, P IV dan P V, diinfeksi dan diobati dengan 17 ppm ekstrak daun *A. annua* (P VI) dan diinfeksi dan diobati dengan Sulfa (P VII). Setiap ayam diinfeksi dengan *E. tenella* dosis rendah, yaitu 2000 ookista kecuali kelompok ayam normal. Pengobatan dilakukan setiap hari selama delapan hari secara oral. Variabel yang diamati adalah gejala klinis, jumlah ookista dalam feses, pertambahan bobot badan, skor perlukaan sekum, nilai hematokrit (PCV) dan hemoglobin (Hb). Hasil penelitian menunjukkan bahwa pemberian 17 ppm ekstrak daun *A. annua* (P VI) paling efektif dalam menurunkan jumlah ookista (74,18%) diikuti dengan perlakuan 34 ppm artemisinin (58,14%). Ekstrak daun *A. annua* dan artemisinin juga mampu mengurangi perlukaan sekum secara nyata (P<0,05). Walaupun pertambahan bobot badan dan nilai Hb juga tidak menunjukkan perbedaan yang nyata pada semua perlakuan (P>0,05), tetapi pemberian artemisinin dan ekstrak daun *A. annua* mampu mempertahankan nilai PCV secara nyata dibandingkan P II dan P IV (p<0,05) sehingga dapat digunakan sebagai antikoksidia alternatif untuk ayam.

Kata kunci: *Eimeria tenella*, Artemisinin, *Artemesia annua*, Koksidiosis

ABSTRACT

Wiedosari E, Wardhana AH. 2017. Anticoccidial activity of Artemisinin and Extract of *Artemesia annua* leaves in chicken infected by *Eimeria tenella*. JITV 22(4): 196-204. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1622

The continuous use of anticoccidial drug in chicken often continuously generates drug resistance and tissue residue; so thatconsequently, a safe alternative anticoccidial drug based on herb is fundamentally required. The aim of thise study was to examine anticcocidial activity of artemisinin and extract of *Artemesia annua* leaves in chicken infected by *Eimeria tenella*. A total of 35 chickens of Cobb strain was divided into seven groups with five replicates birds per group, i.e. uninfected chicken group (P I), infected but untreated chicken group (P II), infected and treated chicken group with 8.5 ppm, 17 ppm, 34 ppm, for P III, P IV and P V respectively, infected and treated chicken with 17 ppm of *A. annua* extract (P VI) and infected and treated chicken with Sulfa (P VII). All chicken, except the uninfected group, whereas infected with 2000 infective oocyst of *E. tenella* except the uninfected group. Treatment was delivered by oral, once per day for eight days. The criteria observed were clinical manifestation of chickens, number of oocyst in feces, body weight, cecal lesion score, haematocrit (packed cell volume) and haemoglobin value. The results showed that extract of *A. annua* leaves (P VI) was the most effective treatment to reduce the number of oocyst in feces (74.18%), followed by 34 ppm of artemisinin group (P VII). In addition, application of *A. annua* extract and artemisinin was significantly able to decreased the cecal lesion score (P<0.05). Even though body weight and Hb value were not indifferent significantly different (p>0.05), however *A. annua* extract and artemisinin treatments were significantly able to hold PCV value on normal level compared to P II and P IV (P<0.05). It concluded that extract *A. annua* leaves and artemisinin could be used an alternative anticoccidial in chickens.

Key Words: *Eimeria tenella*, Artemisinin, *Artemesia annua*, Coccidiosis

INTRODUCTION

Coccidiosis in chicken remains a major problem in poultry industry in Indonesia and many other countries. This disease is caused by intracellular protozoa, Eimeria spp, which is highly pathogen and able to multiply at 21 – 32°C and 75-85% humidity. Infection of the *E. tenella* in poultry injures intestinal mucosae which results in decreases nutrient absorbtion and feed eficiency, and increase secondary bacteria infection (Morris et al. 2007; Cooper & Songer 2009). Adamu et al. (2013) showed that *E. tenella* infection in chicken caused decreases in hematocryte (packed cell volume, PCV) and hemaglobin (Hb) that led to the enamia. The annual economic loss caused by the disease is estimated to be 1.5 billion US dollars in the United States alone, or 3 billion US dollaarrs worldwide (Dalloul & Lillehoj 2006; Lee et al. 2009). Tresnani et al. (2012) reported that the morbidity of coccidiosis in Indonesia was 80-90% with the loss reached 70% consisting of decrease body weight, delay in laying, decrease egg production, and decrease in feed efficiency, incraese operational cost (Dalloul & Lillehoj 2006; Wiedosari et al. 2014).

So far, the control of coccidiosis relys on coccidiostate of sophonamide group such as sulfaquinoxalin, sulfadimetoxin, amprolium and decoquinate (Tabbu 2006). However, intensive use of the coccidiostate in the feed reported to induces resistance to *E. tenella* and coccidiostate residue in the poultry products (El-Sadawy et al. 2009; Kheirabadi et al. 2014). This problem could be overcome by rotating the use of coccidiostate with different active substances. Unfortunately, this strategy will raise the production cost of the poultry industrial, and therefore this strategy is rarely used (Abbas et al. 2010). Herbal-based-anti coccidia are an attractive analternative because it does not leave residue in the products or induce resistance to the protozoa. In addition, herbal-based-anti coccidia is compatible for the medication of sub clinical infection or infection with low number of *E. tenella* (Habibi et al. 2016).

Plant of the Artemisia genus was reported to have antiprotozoa activities (Aryanti et al. 2006; del Cacho et al. 2010). The use of A. Sieberi was reported effective for *E. tenella* and E. acervulina but not for E. maxima (Arab et al. 2006). Compared to the A. sieberi, *A. annua* is available more abundantly, and had been used widely for treatment many parasitic diseases. Artemisia annua contains complex terpenoid compounds, including sesquiterpenes lactone compounds known as artemisinin. This plant has been used as an alternative treatment for malaria and in addition it has anti-bacterial, anti-protozoa anti-inflammatory, and anti-tumor activities (Ferreira et al. 2011). A methanol exctract of the *A. annua* leaf and its powder proved to ehance humoral and cellular immune system of broiler chickens (Gholamrezaie et al. 2013). Synthetic artemisinin at 17 ppm concentrations was able to block the formation of ookista walls and prevent the occurrence of *E. tenella* sporulation (del Cacho et al. 2010). Addition of 20% powdered leaf of *A. annua* in feed was reported to increase egg production and the colour intensity of the yolk (Brisibe et al. 2008).

(Drăgan et al. 2014) found that the effectiveness of herbal-based anticoxidia is influenced by several factors such as plant parts used, seasonal variations, herbal drying methods, herbal concentrations, strain and dose of *E. tenella*, application route of anticoxidia, poultry strains. The A-annua leaves dried air-dried- have higher artemisinin content than freeze- dried- methods (Ferreira & Luthria 2010). The objective of this study was to investigate the antikoksidia activity of synthetic artemisinin and *A. annua* leaf extract in Cobb strain chicken infected with *E. tenella* of local isolate at low doses or sub-clinical infections.

MATERIALS AND METHODS

Plant collection and extraction

Leaves of *A. annua* was obtained from the Indonesian Spice and Medicinal Crops Research Institute (BALITTRO) at Lembang, West Java. The leaves were dried at room temperature for 1 week.

The leaves were first macerated, and as much as 300 grams of the leaf powder was soaked in 3 L petroleum ether in Erlemeyer and agitated for 2 hours. The petroleum ether solution that contained the active compound was filtered and evaporated. The *A. annua* leaf extracts which appered as thickly paste was stored in 4°C until used.

Artemisinin

Synthetic artemisinin used in this study was Artemisinin 99% powder (parchem). Artemisinin powder was dissolved in sterile distilled water until used.

Experimental animal

Day old Cobb broiler chicks were kept in a cage that previously had been decontaminated with potassium permanganate ($KMnO_4$) and 40% formalin solution at a ratio of 1 : 2. Vaccination against New castle disease was given when the chicks was at 1 week old. The birds are fed on pelleted ration that did not contain any coccidiostat. Drinking water was given ad libitum.

Isolation and propagation of *E. tenella*

Eimeria tenella used in this study was isolated from infected chicken in Sukabumi. The contents of the cecum were removed, suspended in solution of Sheather sugar, filtered and carified by centrifugation. The presence of oocysts in the supernatant was examined under a microscope. The *E. tenella*, after confirmed by its morphology and size was isolated. The parasites were passaged in six days old, free-coccidia chicks. The oocysts were put into a Petri dish, potassium bichromate (K2Cr2O7) at a concentration of 2.5% was added, and incubated at room temperature for three days in a slightly opened Petri dish. Sporulated oocysts were used to infect by experimental chickens (Jiang et al. 2012; Khaier et al. 2015).

E. tenella infection

Prior to experimental infection, the oocysts were washed in distilled water and inoculated orally to chicken at a dose of 2000 sporulated oocysts per bird. The chickens were put into a cage based on the treatment group. Clinical symptoms and the occurrence of blood defecation observed until day eight.

Treatment

Thirty five chickens were divided into 7 groups, 5 birds/ group. Following was the groups and treatment applied:

PI : Uninfected
PII : Infected but untreated
PIII : Infected and treated with 8.5 ppm of arteminisin powder
PIV : Infected and treated with 17 ppm of arteminisin powder
PV : Infected and treated with 34 ppm of arteminisin powder
PVI : Infected and treated with17 ppm of *A. annua* leaf extract
PVII : Infected and treated with sulfonamide compound (sulfadiazine 200 mg)

The treatments were performed once a day since the first day after *E. tenella* infection orally for eight days.

Observation of caeca lesion

Necroption was performed eight days after infection. All abnormalities on the cecum were recorded and the damage on the mucosal surface was scored, from scor 0 to 4 (Tabbu 2006). Score 0 was for normal or no lesion; score 1 was mild lesion, ptechiae spread on the surface of the caecal mucosa with slight changes in wall color or contents of the gastro intestinal tract (cecum); score 2 was moderate lesion characterized by more severe haemarrhage and a slightly thickening of the cecum wall; score 3 indicated severe haemarrhage with blood clots in the caeca lumen, and; a score of 4 indicated very severe lesions characterized by severe, widespread haemarrhages, blood clots in the lumen and bluish-red coloring of the caeca walls.

Oocyst Excretion

This examination was carried out daily on several birds. One gram of chicken feces or litter was suspended in 29 mL saturated salt solution. Then, the suspension was centrifuged at 1500 rpm for 10 minutes. The supernatant was loaded into a McMaster's chamber and the number of oocyst was counted under a microscope at 400 x magnification. The total number of oocysts in both boxes of the Mc Master chamber was counted and then divided by two. The average oocyst yield is multiplied by 200 to obtain the number of oocysts per gram of feces (Khaier et al. 2015).

Body Weight Gain and Haematology

Birds were weight daily from the day of infection until the day of necropsies. Examination of hemoglobin (Hb) was performed by Sahli method using Haemoglobinometer (Ogbe et al. 2010). Measurement of hematocrit values (packed cell volume, PCV) was performed according to previous method (Ogbe et al. 2010).

Statistical Analysis

A Completely Randomized Design with 7 treatments was used. Data on body weight gain and oocysts excretion were analyzed by analysis of variance (ANOVA) while the data of caeca lesions were analyzed using Kruskal Wallis. When significant differences in the means were found, the smallest real differences test was carried out. For the Hb and PCV, the values were analyzed descriptively by comparing values with normal reverence values.

RESULTS AND DISCUSSION

Artemisinin in *A. annua* is synthesized in the root and accumulated in leaves and other plant parts. Artemisinin content in leaves is the highest which may reaches 89% of the total content of plants (Laughlin 2002). Based on HPLC-UV analysis, (Drăgan et al. 2014) reported that *A. annua* leaf contained 0.75% artemisinin (Art), 0.18% dihydroartemisinic acid (DHAA) and 0.03% artemisinic acid (AA). The buds and flowers contained only 0.2% artemisinin (Art) and 0.3% dihydroartemisinic acid (DHAA) (Dragan et al. 2013). Brisibe et al. (2009) successfully identified bioactive compounds in *A. annua* as flavanoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids and sesquiterpenoids. In addition, *A. annua* leaves also contain many proteins, essential amino acids, minerals, vitamins and antioxidants. For this reason, synthetic artemisinin was included as control for the *A. annua* leaf extract.

Clinical symptoms observation

Chickens in the control group (PI - without infection) showed no sign of disease or abnormality. The infected groups showed panting (breathing rapidly through the mouth), lethargy, but their appatide seemed unaffected. Jatau et al. (2014) found that chickens infected with low doses of *E. tenella* show only mild clinical symptoms such as decreased activity and appetite and mild diarrhea.

Hematochezia or bllody stool was observed at fifth day after infection. This is in accordance to the observations of Jatau et al. (2014) which showed that generally a blood defectaion occured on the third or fifth day after infection of *E. tenella* but the the haemarrhage is short lasting. The condition is usually observed in mild infection of *E. tenella* which causes non-fatal, mild clinical symptoms. Ogbe et al. (2009) reported that the incidence of blood defecation in chickens infected with high-dose, 20000 oocysts of pathogenic strain occurred on the fifth day and followed by death if not treated.

Excretion of Oocysts in Feces

The appearence *E. tenella* oocysts in feces was first detected in five days after infection concurently with blood defecation. This result is in accordance with that of previous study which reported that *E. tenella* oocysts in feces were detected on the fifth post-infection (Drăgan et al. 2014). Furthermore, Jatau et al. (2014) also observed that the oocysts of *E. tenella* infection in different chicken strains (Marshal and Cobb) also showed the appearance of oocysts in feces on the day five after infection.

The excretion of *E. tenella* oocyst in the present study was principally similar to that of Pop et al. (2015), in which, oocyst excretion peaked at seventh day and waned on eighth day. In general, the peak excretion of *E. tenella* oocysts in infected chickens without treatment (PI) was higher than that in other treatments. However, the intensity of decrease in the oocyst excretion on the eighth day was not the same in all groups. The excretions of oocysts in chickens treated with the synthetic artemisin or *A. annua* extract were lower than that othe groups (P VI, Figure 1).

Observations on the eighth day showed that the decrease in *E. tenella* oocyst excretion had a negative correlation with the concentration of. The higher artemisinin concentration given the lower the oocysts excreted, decreasing from 58.14 to 23.55 - % (Table 1). The decrease was lower than that reported by de Almeida et al. (2012), which was about 60-70% in chickens infected naturally with *E. tenella*.

The highest decrease in oocyst excretion (74.18%) was in the infected chicken treated with *A. annua* leaf extract (Table 1). These results indicate that other compounds may enhance the activities of the artemisinin in in the extract of *A. annua* leaf. Infected chicken treated with comercial suphonamide decreased the oocysts excretion only by 50% (Table 1). However, the decrease in oocysts excretion after A. annua leaf extract treatment as found in this study was lower than that of Dragan et al. (2013) in which chickens infected with 1500 oocyst and treated with A. annua leaf powder reduced the number of oocysts in the feces by 87.9%.The differences are might be to be due

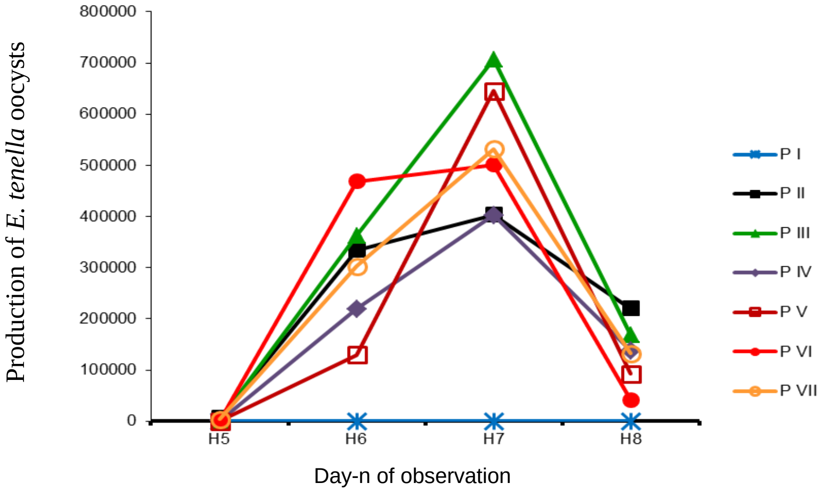

Figure 1. Excretion of oocysts in the feces of chicken five to eight days after *E. tenella* infection.

to the variation in concentration of active compounds in the *A. annua* leaf or differences in the pathogenicity *E. tenella* strain used.

The decraese in *E. tenella* oocysts in chicken feces after the administration of artemisin is also reported by del Cacho et al. (2010). A flow-cytometry analysis revealed that pure artemisinin (99.5%) at 17 ppm dose damaged the cell wall of oocysts as indicated by the uptake of propidium iodide resulting in the death or failure of the oocysts to sporulate. A significant decrease by 24.85% - 36.34% in spontaneous oocyst sporulation amount was observed. This finding may explain the decrease in the excretion of *E. tenella* oocysts found in feces (del Cacho et al. 2010).

Chicken body weight

The body weight gain of chicken is presented in Table 1. The mean body weight gain between groups of chicken were not significantly different (P>0.05). This result indicates that treatment of chicken with artemisinin daily does not significantly affect body weight gain.

This result is in aggrement with Dragan et al. (2013) which compared weight gain *E. tenella*- infected chicken treated with powder of *A. annua* leaves, essential oli of *A. annua* leaves, Tween 80 and cocsidiostat (Lasalocid). The means of weight gain from the age of 7 - 35 days were not significantly different between treatments. As a matter of fact, chicken treated with *A. annua* leaf powder tended to decrease body weight on day 41. Engberg et al. (2012) demonstrated that chickens treated with hexane extract of *A. annua* leaf at a concentration of 500 mg/kg of diet showed no significant body weight gain compared to control treatment.

de Almeida et al. (2012) also reported that there was no significant differences in mean weight gain between chickens fed with *A. annua* leaf and controls. Prior to infection with *E. tenella*, chickens fed with *A. annua* leaf mixture even had a lower body weight gain than controls. The study also showed that the chickens infected with *E. tenella* and treated with commercial anti coccidia sulfonamide had similar body weight gain as that in the group of chicken treated with *A. annua* leaf extract.

Several factors may explain those different results. There is a possibility of variation in the concentration of compound components contained in *A. annua* leaf due to differences in planting season, the differences in the methods of *A. annua* leaf preparation, differences in research design, and differences in the susceptibility Eimeria species used against active compounds of *A. annua* (Drágan et al. 2010; Ferreira & Luthria 2010). Furthermore, de Almeida et al. (2012) explained that artemisinin in *A. annua* causes a bitter taste in the diet leading to the decrease in chicken palatability. Therefore, there is a demand for additional natural ingredients that can reduce bitter taste in feeds such as Stevia rebaudiana leaves or molasses to increase chicken palabilaty that ultimately increases body weight and reduces the production of *E. tenella* oocysts (Kheirabadi et al. 2014).

Table 1. Body weight gain and inhibition oocysts excretion in chicken infected by *E. tenella* treated with artemisinin and *A. annua* leaf extract for eight days

Treatment	Average body weight (g) ± SE		Body weight gain (g)*	Inhibition of oocysts excretion (%)
	Initial	End		
P I	426 ± 9.27	808 ± 26.34	381 ± 22.67; -	-
P II	428 ± 12.00	744 ± 34.87	316 ± 39.06; 00.00	0
P III	450 ± 20.25	860 ± 45.14	410 ± 27.34; 29.75	23.54
P IV	426 ± 42.38	794 ± 41.06	368 ± 62.40; 16.46	37.5
P V	422 ± 25.57	880 ± 24.29	458 ± 27.82; 44.94	58.14
P VI	430 ± 21.08	794 ± 24.82	364 ± 16.91; 15.19	74.18
P VII	432 ± 14.28	797 ± 21.35	364 ± 16.31; 15.19	50.00

* Not significantly difference (P>0.05)

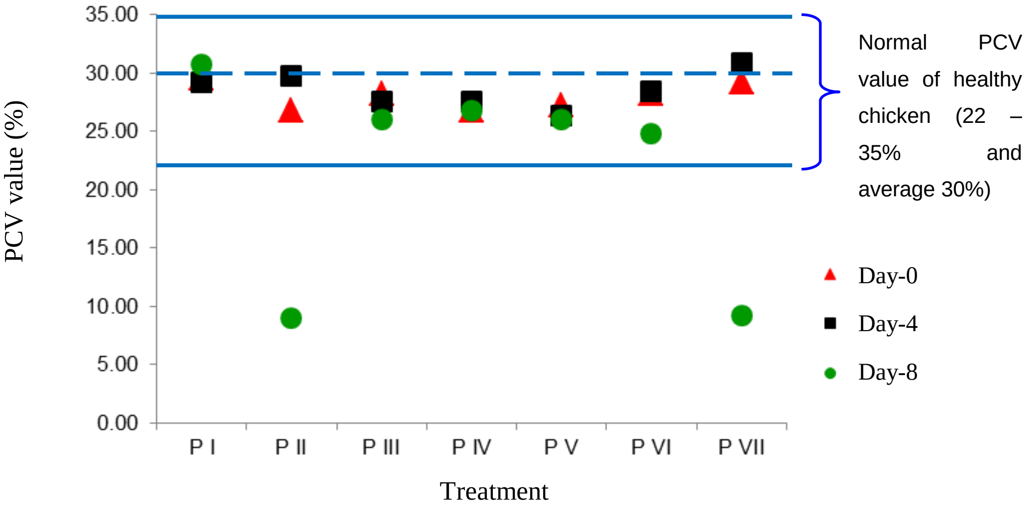

Figure 2. PCV value in chicken infected with *E. tenella* treated with artemisinin and *A. annua* leaf extract.

The unsignificant differences in mean body weight gain as observed in this study were also suspected to be influenced by infectious doses and *E. tenella* oocyst strains and chicken strains. Jatau et al. (2014) demonstrated two different responses between two chicken strains (Cobb and Marshal) against the infection of low doses of oocyst *E. tenella* (3000 oocysts). The average weight gain in Marshal chicken was not affected by low dose of *E. tenella* infection.

PCV (hematocrit) value

Generally, coccidiotic chickens are supposed to develop an anemia due to loss of red blood cell (Jatau et al. 2014). The PCV values on the 0 day of observation in this study showed normal values in all treatment groups (26.20 - 29.60%). A significant decrease was found on the eighth day, where the PCV value of chickens infected with *E. tenella* without treatment (P II) and chickens infected with *E. tenella* with commercial product treatment (P VII) ranging from 9.00 to 9.20% (Figure 2). Compared to the control (PI), the average PCV value decreased in both group by 70-70.8%.

These results indicate that administration of synthetic artemisin and *A. annua* leaf extract for eight days was able to maintain PCV values from *E. tenella* infection. Similar results had been observed in previous studies (Jatau et al. 2014; Ogbe et al. 2010).

Hb (haemaglobin) level

The Hb level in four and eight days after infection of different groups of chicken are presented in Figure 3. In general, Hb levels in all treatment groups decreased from the fourth day to the eighth day, but the reduction was statistically insignificant (P>0.05) and still in the normal value for chicken, 9.00 - 11.50 g / dL.

Phatology-anatomy observation of chicken cecum

Pathologically, no lesion was found in the negative control group (PI). In the infected-untreated group (P II), the lesion scored to 3.6 (severe tissue damage, thickening of mucosae, hemorrhage and calcification in wide areas). The infected-treated chicken group (P II - P VII) displayed less severe lesions, scored to 1.6-2.2. The changes include red spots or ptechia scattered in the caecal mucosa. Administration of synthetic artemisinin and *A. annua* leaf extract in chickens had an effect in preventing tissue damage (Table 2).

In this study, the percentage of scores of caecal damage in chicken treated with *A. annua* leaf extract (44%) was lower than that in chicken treated with synthetic artemisin (61-72%). However, it is more effective than that reported in a previous study. Dragan et al. (2010, 2014) reported a percentage score of 56% on low infection (1,500 ookista) and 56% in high infection (10,000 oocysts). Other study in chickens infected naturally with *E. tenella* and treated with powder and leaf essential oils of *A. annua* displayed caecal damage score of 58% and 68% , respectivelly (Dragan et al. 2013). This mild caecal damage in the treatment group, presumably because the active compound of artemisinin has high antioxidant content and strong anti-inflammatory properties to inhibit *E. tenella* infection (del Cacho et al. 2010).

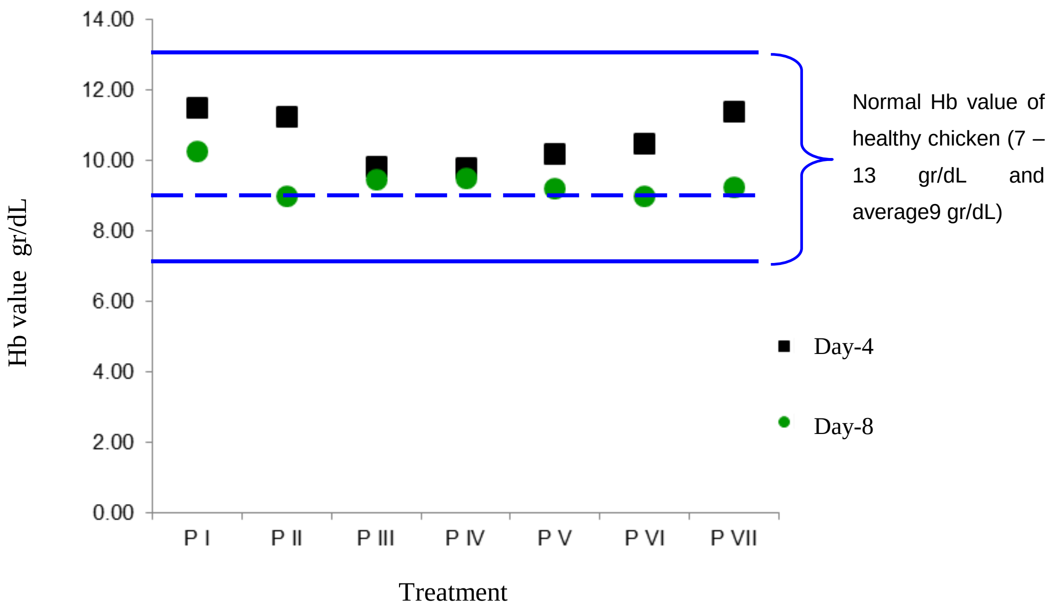

Figure 3. Hb value (gr/dL) in *E. tenella*-infected chicken treated with artemisinin and *A. annua* leaf extract.

The mechanism of artemisinin against *E. tenella*

There are at least two mechanisms of artemisinin compounds ,directly at the developmental stage of parasites and indirectly through micro floral interactions in the digestive tract (de Almeida et al. 2012). The later is supposed to prevent activation of proinflammatory factor by the parasite and the body's immune response to *E. tenella* infection. Although administration of *A. annua* does not remove parasites completely, it may improve the immune system and resistance to infection, including lowering the risk of secondary bacteria infection. The anti-parasitic mechanisms of artemisinin compounds is hypothesed to be associated with the process of ookista wall formation resulting in the death of oocytes ultimately decreases the number of oocytes excretion (del Cacho et al. 2010). This mechanism involves inhibition expression of SERCA (sarco / endoplasmic reticulum calcium ATPase) in macrogametes that play an important role in calcium homeostasis. This disturbance inhibits the formation of oocytes walls which ultimately leads to oocytes death. Titilincu et al. (2008) demonstrated that suspension of oocytes on medium containing *A. annua* inhibit sporulation processes and damage sporulated oocytes.

Prevention and treatment of coccidiosis in chickens with artemisinin is relatively safe and does not affect weight gain, feed and water consumption as long as given at the right dose (Shahbazfar et al. 2011) According to Kheirabadi et al. (2014), artemisinin is relatively slow to absorb the body which is advantageous for treatment of coccidiosis. The slow absorption allows prolong interaction of the active compound with parasites in the digestive tract. Other advantage of applying artemisinin is its capacity to reduce oocytes contamination in the litter cage.

Table 2. Score of caecal lesion in *E. tenella*-infected chicken treated with artemisinin and *A. annua* leaf extract

Treatment	Lesion score	Average *	%
P I	0, 0, 0, 0, 0,	0^{a}	0
P II	+4, +4, +3,+3, +4	3.6^{b}	100
P III	+3, +3, +3,+2, +1	2.4^{c}	66.67
P IV	+3, +3, +2,+2, +1	2.2^{c}	61.11
P V	+3, +2, +2,+2, +1	2^{c}	55.55
P VI	+3, +2, +1,+1, +1	1.6^{c}	44.44
P VII	+3, +3, +2,+2, +1	2.2^{c}	61.11

* Values with different superscript in the same column shows significantly difference (P<0.05)

CONCLUSION

The *A. annua* leaf extract and synthetic Artemisinin are effective in reducing the severity of caecum lesion and depress oocyst excression in feces in chicken infected by *E. tenella*. Extract of *A. annua* leaf that contains artemisinin can be used as an alternative to commercial sulfonamides.

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Contamination of *Escherichia coli* O157:H7 in Dairy Cow Farms

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ABSTRAK

Rachmawati F, Ariyanti T. Kontaminasi bakteri *Escherichia coli* O157:H7 pada peternakan sapi perah. JITV 22(4): 205-211. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1674

Bakteri *E.coli* O157:H7 adalah foodborne pathogen yang membahayakan kesehatan manusia. Ternak sapi merupakan reservoir utama dari bakteri ini. Bakteri *E.coli* O157:H7 yang keluar dari ternak kemudian dapat mengkontaminasi lingkungan sekitar, produk susu, daging, tanaman sayur-sayuran ataupun buah, sehingga dapat berperan sebagai sumber infeksi dan mengakibatkan penyakit pada manusia. Penelitian ini dilakukan untuk identifikasi adanya kontaminasi bakteri *E.coli* O157:H7 pada peternakan sapi perah sehingga diketahui situasinya terkini. Pengambilan sampel untuk penelitian ini adalah peternakan sapi perah di wilayah Depok, Cianjur, Sukabumi dan Bandung. Jenis sampel yang diambil yaitu ulas dubur sapi atau feses segar dan air minum ternak. Isolasi bakteri *E.coli* dilakukan pada media mTSB, MacConkey Agar, Eosin Methylene Agar. Identifikasi bakteri sebagai *E.coli* O157:H7 dilakukan dengan uji biokimia dan untuk penetapan serotipe digunakan uji agglutinasi menggunakan antiserum monospesifik untuk O157 dan H7. Hasil penelitian memperlihatkan bahwa sampel kotoran dan air minum sapi dari seluruh peternakan sapi perah yang diteliti telah terkontaminasi oleh bakteri *E.coli*. Dari 178 sampel yang diuji telah diidentifikasi sebanyak 126 (70,8%) sampel diantaranya mengandung bakteri *E.coli*. Dari jumlah 126 sampel yang mengandung bakteri *E.coli*, ternyata sebanyak 94 (74,6%) adalah bakteri *E.coli* serotipe O157:H7. Keberadaan kontaminan bakteri *E.coli* O157:H7 pada peternakan sapi perah, potensi dampaknya bagi kesehatan manusia, serta upaya pencegahan dan pengendalian bakteri ini dibahas dalam tulisan ini.

Kata Kunci: Sapi Perah, *E. coli*, *E. coli* O157:H7, Lingkungan

ABSTRACT

Rachmawati F, Ariyanti T. Contamination of *Escherichia coli* O157:H7 in dairy cow farms. JITV 22(4): 205-211. DOI: http://dx.doi.org/10.14334/jitv.v22i4.1674

E. coli serotype O157:H7 is a foodborne pathogen that endangers human health. Cattle are the main reservoir of this bacterium. *E. coli* O157:H7 that come out of the livestock can contaminate the surrounding environment, dairy products, meat, vegetable or fruit crops, so it can act as a source of infection and disease in humans. This research was conducted to identify the contamination of *E. coli* O157:H7 on dairy cow farms so the current situation is known. Sampling area for this research was dairy cow farm in Depok, Cianjur, Sukabumi, and Bandung. Types of samples taken are cow anal swab or fresh feces and livestock drinking water. Isolation of *E. coli* bacteria was done on mTSB media, agar MacConkey, and Eosin Methylene Agar. Identification of bacteria as *E. coli* O157:H7 was done by biochemical test and for serotype determination was used agglutination test using monospesifik antiserum for O157 and H7. The results of research showed that of the total 178 samples had 126 (70.8%) samples containing *E. coli* bacteria. Of the 126 samples containing *E. coli* bacteria, 94 (74.6%) were determined as *E. coli* serotype O157:H7. The presence of *E. coli* O157:H7 contaminants on dairy cow farms, their potential impacts on human health, prevention and control of the disease is discussed in this paper.

Key Words: Dairy Cow, *E. coli*, *E. coli* O157:H7, Environment

INTRODUCTION

Escherichia coli (*E. coli*) is a bacterium that lives normally in the digestive tract of animals and humans. *E. coli* is one of the bacteria belonging to the family of Enterobacteriaceae in the form of a straight, single, paired or short chain rod, measuring 2.0-6 µm in length and 1.1-1 µm in diameter. This bacterium is a Gram-negative, motile or non-motil, aerobic or anaerobic facultative group. *E. coli* bacteria were first discovered by Theodore Escherich in 1885 (Scheutz & Nancy 2005).

E. coli O157:H7 is one of the *E.coli* serotypes belonging to the pathogenic Enterohaemorrhagic *Escherichia coli* (EHEC) group. The cow is an EHEC reservoir, and the bacteria can contaminate the environment as well as its contacts with feces. This bacteria is associated with outbreak of foodborne pathogen and sporadic cases in humans in the world (Karmali et al. 2010; Rangel et al. 2005).

The typical symptoms of EHEC infection in livestock and humans are bloody diarrhea. Bleeding diarrhea as a results from verotoxin or Shiga Like Toxin (SLT) produced by EHEC, especially serotype O157:H7. This serotipe was first isolated in 1982 in the United States, during an outbreak of haemorrhagic colitis (HC) in Oregon and Michigan (Bonardi et al. 1999). This serotype is often called verotoksigenik *E. coli* (VTEC). Verotoksin is extracellular, neurotoxic and immunogenic (Ball et al. 1994; Pirro et al. 1995). Human EHEC infection results in haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) were also reported (Konowalchuk et al. 1977). Since it was first isolated, the EHEC infection that caused an outbreak of food poisoning was reported as a result of consuming milk, beef, vegetables and fruits contaminated by *E. coli* O157:H7. In the United States the disease is the second order after Salmonella infection (CDC 1997; Karmali 1989; Hara-Kudo et al. 2000).

In Canada, there was human HUS case as a result consumption of milk in a dairy farm in Canada in 1986. The *E. coli* O157:H7 was isolated from young cow feces in that dairy farm. Other studies showed that *E. coli* O157:H7 has been isolated from faecal samples from 16 head of cattle or calves and 1 dairy cow from 22 farms, 1 calf from fattening cattle; 2 samples of beef, and 1 milk sample (Wells et al. 1991). Bonardi et al. (1999) reported that the VTEC O157 strain was isolated from faecal samples collected from one of the slaughterhouses in Northern Italy, faecal samples from 59 head of cows, faecal sample from 450 cows of 133 farms, 37 cows from 223 fattening places, 22 from 137 dairy cows that were derived from 60 farms.

In Indonesia, the presence of EHEC bacteria was reported in dairy calves in Bandung, Sukabumi, Cianjur, and Bogor, with symptoms of bloody diarrhea. The bacteria were identified belong to the VTEC O157 group that lyses vero cells (Kusmiyati & Supar 1998), However no further identification whether the bacteria as serotype O157:H7. Suwito (2009) identified the presence of this *E. coli* O157:H7 in milk samples from dairy cow farms in Bogor, Sukabumi and Cianjur districts. Other studies reported that *E. coli* O157:H7 was detected as bacterial contamination of beef, fresh milk, pasteurized milk, crabs, white shrimp, bottled water and refills (Sartika et al. 2005; Suardana et al. 2007; Kandau 2009; Marlina et al. 2009. Aryanti & Maryam (2015) also reported that of 120 samples consist of meat, fresh milk, beef meatballs, beef jerky, sausage, abon, UHT milk, yoghurt and cheese, 39 (32%) of those contained *E. coli* O157:H7. Suardana et al. (2016) reported 5 (8.6%) of 58 samples of cow feces in seven villages in Kecamatan Petang, Kabupten Badung, Bali were positive for *E. coli* O157:H7.

This research was conducted to identify the contamination of *E. coli* O157:H7 bacteria on dairy cow farms so the current situation is known to minimize risk to human health.

MATERIALS AND METHODS

Bacteria of reference

Bacteria used as a reference in this study were *E. coli* O157:H7 ATCC 43984.

Collection of samples

Samples, swab of anal or fresh feces and drink water of cow, were collected from dairy farms in Depok, Cianjur, Sukabumi and Bandung areas of West Java Province. The feces were taken using sterile cotton swabs and then were inserted into a sterile bottle containing bacto pepton water (transport medium). Sample of drinking water was taken from the pot and put it in sterile plastic bag. All samples were stored in an ice box and transported to the Bacteriolgy Laboratory, IRCVS Bogor.

Isolation and identification of *E. coli*

Isolation of *E. coli* O157:H7 followed the procedure of BSN (2008) with modification, i.e 1 gram of feses, or 1 ml of cattle drinking water added with 9 ml mTriptic Soy Broth (mTSB) which was added Novobiocin, then incubated for ± 24 hours at 37˚C. Furthermore, the culture was grown on MacConkay Agar or Eosin Methylene Blue (EMB) agar medium at 37°C for ± 24 hours. The alleged colony of *E. coli* will indicate lactose or pinkish pink fermentation on MacConkey media Agar or metallic green in EMB. Furthermore, Gram staining is done, planted it in identification media such as Triple Sugar Iron (TSIA), semisolid media / motility test and Indol test, Methyl Red, Voges Prouskauer, Simmon's Citrate (IMViC). After the bacteria was identified as *E.coli*, the culture was subsequently grown on Sorbitol MacConkay Agar (SMAC) added with Cefixime-Tellurite selective supplementation according to Oxoid Microbiology

Product procedure, then incubated at 37°C for 16-20 hours. Confirmation of serotype *E. coli* O157: H7 was performed by serotyping it using a monospecific O157 and H7 antiserum (Bonardi et al. 1999; Barrow & Feltham 2003).

Serotyping isolates as *E. coli* O157:H7

All of the isolated *E.coli* were examined by agglutination test using monospecific O157 and a monospecific H7 antiserum produced from previous studies (Supar et al. 1988). All *E. coli* isolates were grown on Nutrient Agar (NA) medium and incubated at 37˚C for ± 24 hours were then suspended with a physiological NaCl solution with turbidity equivalent to Mc Farland No. 10. Furthermore, the bacterial suspension was heated to a temperature of 100°C for 2 hours or at 121°C for 1 hour, then cooled and checked with a monospecific antiserum O157. Agglutination test was carried out by means of twenty microliters of *E. coli* antigen (sample) mixed with 20 μL monospecific O157 antiserum. The agglutination occure within 1-3 minutes in the form fine clumps like sand.

As for examination of somatic antigen H7, all *E. coli* isolates were grown on a semisolid medium in a tube equipped with a craigic tubes and were incubated at 37°C for ± 24 hours. Bacterial cells grown outside the craigic tube were grown onto NA medium and incubated at 37°C for ± 24 hours. By using a sterile ose, the culture of *E. coli* on NA medium was taken and placed it on the glass object, then it was added with 20 μL sterile aquades and mixed it with 20 μL monospesifik H7 antiserum for agglutintaion test. The agglutination occurs within 1-3 minutes.

Statistical analysis

Data of microbes identified, percentage and its distribution was descriptively analyzed.

RESULTS AND DISCUSSION

The results of sample collection from dairy cow farms and summary of laboratory testing is presented in Table 1.

To identify contamination status of dairy cow farm with the *E. coli* O157:H7, West Java Province was used for sample collection since the population of dairy cattle in this area is high enough, ie 122.811 (22.5%) of total population 545.000 head of dairy cattle in Indonesia (DGLAH 2017).

Biochemical examination of the bacteria isolated from samples (feces and drinking water) grown on the media for Eosin Methylene Blue and IMViC showed that 126 (70.78%) of 178 samples containing *E .coli*. Number percentage of *E. coli* isolated in feces samples was around 40.40 - 85.18 %, while *E.coli* in drinking water of cow was from 11.11 – 100%. (Table 1). The *E.coli* contamination was found in all dairy cow farm located in all location under studied.

Confirmation test of the *E. coli* isolated into O157:H7 serotype using O157 and H7 monospesific antiserum showed that 94 (74.6%) of 126 *E. coli* isolates were positive as O157:H7. Distribution of location contaminated by *E. coli* O157:H7 serotype and its percentage can be seen in Table 1, indicating that all locations were contaminated by the microbe.

Off the total *E. coli* serotype O157:H7 isolated, 1.06% (1/94) of *E. coli* O157:H7 isolate was from the original faecal samples of dairy cow farms in Depok; as much as 20.2% (19/94) of *E. coli* O157:H7 isolates were from faecal samples of dairy cow farm in Cianjur; 2.12% (2/94) of *E.coli* O157:H7 isolate were obtained from drinking water sample of dairy cow farm in Cianjur, and as much as 34% (32/94) isolate *E. coli* O157:H7 obtained from faecal sample of dairy cow farm in Sukabumi, and 1.06% (1/94) isolate *E. coli* O157:H7 obtained from drinking water of dairy cow farm. Isolates of *E. coli* O157:H7 obtained from the Bandung area were 31.9% (30/94) of faecal samples and 1.06% (1/94) of the drinking water in the farm.

The number and percentage of *E. coli* O157:H7 identified from *E. coli* isolated from the feces samples of its size varied between 14.28 - 100% (Table 1, Figure 1). While in drinking water for livestock on farms the contamination may reach 100% (Table1, Figure 2).

In this study, the percentage of *E. coli* O157:H7 identified in dairy farms is quite high from faecal samples. In drinking water of the dairy cow the microbes extracted from 100% of the farms. This data may indicate that majority of dairy cow farm in Indonesia seem to be infected with *E. coli* O157:H7.

Based on the results of this study, shows that all dairy cow farm in all location has been found the existence of *E. coli* O157:H7, both in feces and drinking water of dairy cow. *E. coli* O157:H7 found in the feces potentially may contaminate the environment around farms, such as drinking water pot, equipment, soil, grass, milk udders, milk, other healthy cow, or technician who operate the pens, or the farmer and family member itself, so that horizontal transmission can occur.

Table 1. Results of isolation of *E.coli* and its identification as *E.coli* O157:H7 derived from dairy cow farm in the research areas

Areas/ Location	Farm	Kind of Sample	Number	Laboratory Results			
				Number Positive *E.coli*	%*	Number Positive *E.coli* O157:H7	%**
Depok	Dairy Cow	Feces	10	7	70.0	1	14.28
Cianjur	Dairy Cow	Feces	47	19	40.4	19	100
		Drinking water	7	2	28.57	2	100
Sukabumi	Dairy Cow	Feces 9	54	46	85.18	32	69.56
		Drinking water	9	1	11.11	1	100
Bandung (Lembang)	Dairy Cow	Feces	50	39	78.00	30	76.92
		Drinking water	1	1	100	1	100
Total			178	126	70.78	94	74.60

* Percentage from number of samples
**Percentage of positive *E. coli*

The presence of *E. coli* O157:H7 bacteria in dairy cow feces may also contaminate milk produced by the cow. The milk contaminated with bacteria is very dangerous when consumed by people, when processing of milk is not perfect. The problem of food poisoning caused by drinking milk contaminated with *E. coli* O157:H7 has been reported in Indonesia (Tulung Agung, Bandung and Surabaya) in September 2004 (Kompas 2004). CDC (2007) reported 4 residents in Washington infected by *E. coli* O157: H7 previously becouse they consumed raw cow's milk in December 2005.

Feces of cattle contain *E. coli* O157:H7 and then used it as agricultural fertilizer has great potential to pollute agricultural land and vegetables. As Khandaghi et al. (2010), stated that cow feces are commonly used as fertilizer on agricultural land to be the source of the spread and transmission of *E. coli* O157:H7 due to direct contact or consume unprocessed agricultural produce. From a total sample of 282 soil and vegetable samples such as lettuce, cabbage, carrots and radishes, showed 5 soil samples (1.77%) and one vegetable sample (0.35%) contaminated with *E. coli* O157:H7 bacteria confirmed by multiplex PCR technique.

Ruminants are a natural host of *E. coli* O157:H7, and adult animals infected by the microbe do not show clinical symptoms. Animals infected by the disease after recovery will become a career. Younger animals are more sensitive than adults. Cow as the main reservoir *E. coli* O157:H7 for humans. Although this bacteria can be isolated from other animals with subclinical symptoms, but it is pathogenic for humans. Infected cattle with *E. coli* O157:H7 can excrete these bacteria in a short time or in a long time. *E. coli* O157:H7 can be transmitted through fecal-oral. The spread can be in direct contact between animals, through water resources, feed, or grassland (OIE 2008; CFSPH 2016). Stanford et al. (2005) mentioned that the incidence of mastitis in cow in dairy farms increased due to contamination of *E. coli* O157:H7 contained in the feces of infected cows. So it can be mentioned that cow feces is the main source of contamination of *E. coli* O157:H7 in the environment. Feces of cow as the main source of pollution should be feces waste water flowed into the river, because it can pollute the environment with a wider area. This is very dangerous for humans and other animals. It is possible to have faecal effluent on dairy farms with remote locations with water sources.

The high percentage of *E. coli* O157:H7 in farms due to poor sanitary management of the stable. These bacteria in cows do not show clinical symptoms but are pathogenic in humans. So that its existence will have the potential to spread to the environment around the farm and the possibility of infecting very large other animals and even humans. Although there have been no reports of human illness due to the infection of *E. coli* O157:H7, it has been reported that this bacteria was isolated from humans undergoing dialysis due to kidney failure in Jakarta. While Karawang district, *E. coli* O157: H7 was isolated from patients with diarrhea symptom (Ariyanti 2016).

Prevention of the spread of *E. coli* O157:H7 bacteria needs to be done, to prevent the occurrence of foodborne disease in humans. Control of *E. coli* O157:H7 infection can be applied along the food chain from pre-harvest to post-harvest. Strategy on pre-harvest can be done by reducing the shedding of E. coli O157.H7 in livestock, especially ruminants or by increasing cow resistance against infection of *E. coli*

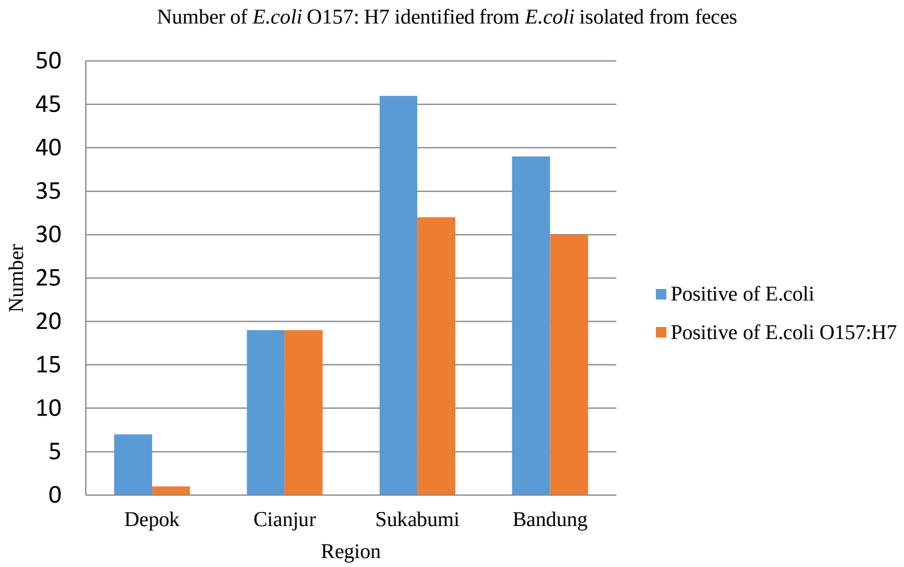

Figure 1. The number of *E.coli* O157:H7 identified from *E.coli* isolated from feces

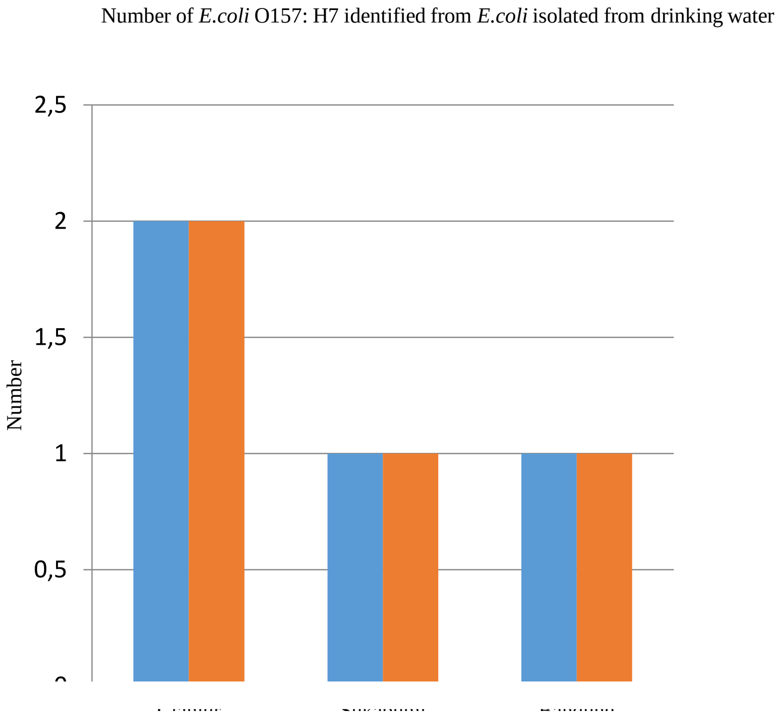

Figure 2. Number of *E.coli* O157:H7 identified from *E.coli* isolated from drinking water

O157:H7 on ruminants in farms correlates with reduction of carcass contamination by the microbe in slaughterhouses. This step shows that interventions at the pre-harvest stage are important and effective in controlling foodborne infection in humans (Ayscue et al. 2009; OIE 2008). The strategy of shedding control in cattle is still being developed (OIE 2008).

CONCLUSION

The fairly high percentage of dairy cow farm contamination with pathogenic *E.coli* O157:H7 have been identified in this studied. This information may indicate that major protion of dairy cow farms could be contaminated by these bacteria. When the bacteria found in the feces, the bacteria may found also in drinking water of cattel in the farms. The presenst *E. coli* O157:H7 in the cow farm has a potential risk to human health through contamination to wider environments such as soil, grass, drinking water source (well), pons, ditch, vegetables, or fruits planted in surrounding contaminated cow farm areas. Prevention and control of *E.coli* O157:H7 infection should always be promoted such as improvement management of animal health and healthy culture in the community. Further research needs to be done is to test the sensitivity of microbes to antibiotics.

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Key Words Index

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UDC: 598.412

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Performa itik pedaging EPMp yang diberi pakan dengan berbagai kadar asam amino lisin sampai umur 10 minggu (Performance of EPMp broiler ducks feed with various levels of dietary lysine up to 10 weeks of age)

(Org: Eng)

JITV 22(1): 1-8

Determining the optimum level of lysine in the ration will be a significant effort to avoid poor growth in duck husbandry. The purpose of this study was to determine the optimum dietary lysine requirement for EPMp broiler ducks, raised up to 10 weeks of age. The study was designed in a completely randomized design (CRD) with 4 dietary treatments, and 4 replicates. Each replicate consisted of 10 ducks. The 4 treatments were diets, containing lysine: 0.60% (T1); 0.70% (T2); 0.80% (T3) and 0.90% (T4). Variables measured included: feed consumption, body weight gain, feed conversion ratio (FCR), carcass and carcass cuts percentages. The results showed that the performance of 10 weeks of age EPMp broiler duck was significantly affected by the level of dietary lysine ($P<0.05$). The average body weight gain and the FCR of the duck were significantly affected ($P<0.05$) by dietary lysine level, but not for feed consumption and percentage of carcass ($P>0.05$). The average body weight gain of EPMp duck with T4 treatment (0.90% lysine) was significantly higher than that T1 (0.60% lysine) but did not significantly difference ($P>0.05$) with T2 and T3. The average FCR of EPMp duck under T3 and T4 were significantly ($P<0.05$) higher than that of EPMp duck under T1. It was concluded that the optimum dietary lysine to produce maximum body weight gain of EPMp duck raised up to 10 weeks of age was 0.70%, while for minimum FCR were at the level of 0.80% and 0.90%.

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Key Words: EPMp Broiler Duck, Dietary Lysine

UDC: 636.2.033

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Analisis pola variabel eksogen terhadap tingkat adopsi teknologi sapi perah di Sumatera Barat (Path analysis of exogenous variables against technology adoption levels of dairy cattle in West Sumatera)

(Org: Eng)

JITV 22(1): 9-15

Path analysis of the regression equation can be used to see the direct and also indirect influence of some exogenous variables against endogenous variables. The rate of feed technology adoption as an endogenous variables can be directly or indirectly influenced by some exogenous variables. The purpose of this research was to test multiple exogenous characteristics variables of dairy cows farms against the feed technology adoption rate as endogenous variables, through path analysis. Research conducted in the city of Padang Panjang, West Sumatra in particular farmer group of dairy cattle in 2016. Endogenous variable is the level of adoption of feed technology (Z). Whereas the exogenous variables are the level of education (X1), the age of farmer (X3), the amount of cow's lactation (X2), farm scale (Y1) and milk production (Y2). The last two variables are variables which are bridging the influence indirectly. Obtained results showed that only X3 which directly influenced Z, with a value of $\rho Zx3 = 0.834$ and $P = 0.018$. Other variables X1 and X2 partly significantly influenced Y1 and X2 significantly influenced Y2 with value of path coefficient in successively $\rho y1x1 = 0.133$ and $P = 0.040$; $\rho y1x2 = 0.982$ and $P = 0.000$; $\rho y2x2 = 0.841$ and $P = 0.008$. Therefore, there was no special model of causal relationships between the empirical variables X and Y against Z, except the X3 which had structure model $Z = \rho Zx3\ X3 + \rho Z\varepsilon 2 = 0.834\ X3 + 0.217\ \varepsilon 2$. It was concluded that the age strongly influenced the feeding technology adoption. The older the age of farmers, the more difficult for adopting recommended technology.

(Author)

Key Words: Path Analysis, Dairy Cattle, Feed Technology Adoption

UDC: 636.92

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Performa produksi kelinci HyCole, New Zealand White dan persilangan timbal baliknya (Production performance of HyCole, New Zealand White Rabbits and its reciprocal)

(Org: Eng)

JITV 22(1): 16-23

New Zealand White rabbits (NZW) has been known as broiler rabbit that has been well adapted in Indonesia. HyCole rabbits were imported from France that were selected for high

reproduction and growth rate. This study was aimed to evaluate the productivity of HyCole and New Zealand White rabbits and their reciprocal as the basis to develop broiler rabbit which adaptive to tropical climate. Forty heads of doe (PBPB) and 17 heads of buck (PAPA) of HyCole rabbit, and 30 heads of doe and 6 heads of buck of New Zealand White rabbits (NN) were used. The ration was given according to IRIAP standard ration (18 % CP, 2500 kcal ME/kg and 14 % CF). The diet was provided in the morning and the evening, and drinking water was provided ad libitum. Reproductive performance of does (litter size at birth, litter size at wean, mortality and weekly does body weight) and the kit's growth performance (weekly body weight from weaning until the age of 20 weeks) were evaluated. Data were analyzed using the SAS program (SAS 2001). Rabbits growth data were periodically analyzed by Gompertz model (Blasco & Gomez 1993). HyCole rabbit which was bred in Indonesia had production performance better than NZW rabbit and PAN crossbred (HyCole bucks x NZW does) had the potential to be bred as superior rabbit adaptive to tropical climate because they had hybrid vigor of the number of litter size at birth and kit's growth rate.

(Author)

Key Words: HyCole Rabbit, NZW Rabbit, Crossbred

UDC: 636.58

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Estimasi berat karkas dan potongan karkas ayam SenSi-1 Agrinak betina (Weight estimation of empty carcass and carcass cuts of female SenSi-1 Agrinak chicken)

(Org: Eng)

JITV 22(1): 24-29

SenSi-1 Agrinak chicken is Indonesian local chicken that was selected for growth rate for six generations. The aim of this study was to estimate of carcass weight and carcass cuts of female SenSi-1 Agrinak chicken, based on age and live weight. The chicks were reared intensively in colony wire cages and they were slaughtered with Islamic method when they reached age of 5, 8 and 15 weeks. Empty carcass and carcass cuts were weight in fresh. Data were analyzed using correlation and regression analysis method. Results showed that the live weight had a high positive correlation to carcass weight and carcass cuts weight of female SenSi-1 Agrinak chickens aged 5, 8 and 15 weeks. Estimation of live weight, carcass, carcass cuts, gizzard, liver and abdominal fat of female SenSi-1 Agrinak, using mathematical model, showed small value differences (0.09 - 4.43%) from the actual data. It was concluded that female SenSi-1 Agrinak chicken's carcass weight and carcass cuts, could be estimated based on of age in days and live-body weight in gram without slaughtering.

(Author)

Key Words: Female SenSi-1 Agrinak Chicken, Empty Carcass, Carcass Cuts

UDC: 636.58

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Efektivitas konsentrasi DMSO terhadap recovery rate dan viabilitas primordial germ cell ayam Gaok (Effectiveness of DMSO concentration on recovery rate and viability of primordial germ cell of Gaok chicken)

(Org: Eng)

JITV 22(1) 30-37

Recent technological developments to produce germ line chimeras with primordial germ cell (PGC) transfer into the recipient embryo provide an opportunity to conserve and retrieval of chicken genetic resources in complete form. The study was conducted to obtain the most effective DMSO percentage to recovery rate and viability of Gaok chicken PGC after freezing which will later be feasible to be transferred. In this study, the eggs of Gaok chicken were incubated for about 2.5 - 3 days to obtain embryos at stages 14 - 16. Blood retrieval was done through the dorsal aorta using micropipettes under microscope. The procedure of PGC isolation of Gaok chicken with centrifugation gradient was using nycodenz as a substance. Commercially available cryoprotectants (dimethyl sulfoxide = DMSO) were used for PGC freezing. Isolated and frozen PGCs of Gaok chicken were diluted with cryoprotectants containing 2.5; 5 and 10% DMSO in fetal bovine serum (FBS). The recovery rate of 2.5; 5 and 10% DMSO concentration were 36.4; 48.2 and 48 % respectively. The viability of PGC after freezing was significantly higher for 5% DMSO compared with 2.5% DMSO ($P<0.05$), but not different from 10% DMSO. It can be concluded that the concentration DMSO of 5 % was effectve contration in freezing Gaok chicken PGC.

(Author)

Key Words: Cryopreservation, DMSO, PGCs, Gaok Chicken

UDC: 578.5

Sawitri, D.H. (IRCVS, Bogor)
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Variabilitas genetik dari gen ESAG 6/7 Isolat *T. evansi* (Genetic variability of ESAG6/7 gene *Trypanosoma evansi*)

(Org: Eng)

JITV 22(1): 38-50

Trypanosoma evansi as an agent of Surra is one of the crucial parasitic diseases that cause great economic losses in Indonesia. These parasites need iron for growth and propagation phase which is obtained by receptor-mediated uptake of host transferin. The transferrin receptors are encoded by Expression Site Associated Genees (ESAGs).

ESAG6/7 encodes transferrin receptors which reported have different affinities of a different host. The distinction of *T. evansi* pathogenicity is supposed to cause variability in the ESAG6/7 gene. This research was aimed to investigate the variability of genes ESAG6/7 *T. evansi* with different virulence in mice. This research was conducted in two steps: bioassay pathogeneicity in mice and analysis of ESAG6/7 gene sequences. The median survival time of mice was investigated after each group of mice infected by 25 *T. evansi* isolates from buffaloes where its geographically differ. The test results showed a difference of pathogenic virulence on 25 *T. evansi* isolates in mice. Sequence analysis of the ESAG6/7 gene from 25 *T. evansi* isolates origin from Indonesia tended to be homogeneous on the transferrin binding site but there was variability in the hypervariable site. These changes are able to separate high and low virulence of the *T. evansi* isolates. Phylogenetic tree analysis was formed 11 clades of 25 *T. evansi*. High virulence *T. evansi* was included in clades 7 and 10, while low virulence *T. evansi* was included in clade 5 and 11 and the moderate virulence was divided into those four clades.

(Author)

Key Words: *T. evansi*, ESAG6/7 Gene, Variability, Virulence

UDC: 636.085.8

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Suplementasi campuran Zn anorganik dan organik pada kambing Boerka yang diberi pakan pelepah kelapa sawit (Supplementation of inorganic and organic zinc mixtures in feed of Boerka goats fed by oil palm fronds)

(Org: Eng)

JITV 22(2): 51-56

Zinc is an element of many enzymes and hormones having very important physiological functions in the body so that it influences the production and reproduction of animals including the activity of the rumen microflora in degrading fiber in a diet. The aim of this study was to improve the performances of goats offered oil palm fronds based diets through the supplementation of 35 ppm of Zn in the form of inorganic (ZnO) and organic zinc (Zn-methionine). Thirty mature male crossing Boer x Kacang (Boerka) goats were divided into five groups and randomly allocated to one of the five feed treatments as follows: P1: complete feed based on the palm oil fronds (Control), P2: P1 + 35 ppm Zn (100% Zn0), P3: P1 + 35 ppm Zn (75% Zn0 + 25% Zn-methionine), P4: P1 + 35 ppm Zn (50% Zn0 + 50% Zn-methionine), P5: P1 + 35 ppm Zn (25% Zn0 + 75% Zn-methionine). The experiment was conducted in a Completely Randomized Design of six replications. Increasing the proportion of Zn methionine in the mixtures elevated feed consumption, and the highest feed intake was observed in goats received 75% Zn-methionine/25% ZnO. Daily body weight gains was only affected ($P<0.05$) by the 75% Zn-methionine/25% ZnO supplement. The concentration of Zn in the blood increased significantly ($P<0.05$) when Zn methionine was added and it increased steadily as the proportion of Zn methionine greater in the mixtures, but the VFA compositions of the rumen were not affected ($P>0.05$) by Zn supplementation. It is concluded that the performances of goat fed complete diets based on the oil palm fronds could be improved by supplementation of inorganic and organic Zn mixture.

(Author)

Key Words: Goats, Zinc, Supplementation, Palm Oil Fronds

UDC: 599.735.51

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Pencitraan ultrasonografi jaringan parenkim ambing anak kerbau Murrah dan Rawa (Ultrasonography of udder parenchymal tissue of Murrah and Swamp buffalo calves)

(Org: Eng)

JITV 22(2): 57-62

The examination of udder through a parameter such as parenchymal tissue (PAR) and mammary fat pads (MFP) can be used to evaluate health status and the development of mammary gland. This research was conducted to evaluate the PAR and MFP of Murrah (n=4) and Swamp (n=4) buffalo heifer calve udder using brightness-mode ultrasonography transcutaneously. Eight buffalo consisted of Murrah (n=4) and Swamp (n=4) buffalo aged of six months old reared at Indonesian Research Institute for Animal Production, Ciawi, Bogor were used this study. Ultrasonography imaging was performed on the udder nipple for the four quarters: left front, left back, right front, and right back. The ultrasonography image was evaluated and then assessed for score, echogenicity and PAR area on MFP. The results showed that sonogram PAR was aniconic to hypoechoic, while the MFP was hypoechoic to hyperechoic. Even though there was a variation in the four quarters of udder, Murrah buffalo calve had the highest ($P>0.05$) value of score, echogenicity, and PAR area compared to the Swamp buffalo.

(Author)

Key Words: Udder, Buffalo Calve, Udder Parenchymal (PAR), Mammary Fat Pads (MFP), Ultrasonography

UDC: 636.5

Susanti, T. (IRIAP, Bogor)
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Pertumbuhan entog putih lokal periode starter dan grower (The growth local white muscovy growth during starter and grower period)

(Org: Eng)

JITV 22(2): 63-67

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

(Author)

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

UDC: 636.58

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Sartika, T. (IRIAP, Bogor)

Karakteristik kualitatif dan kuantitatif ayam SenSi-1 Agrinak (Qualitative and quantitative characteristics of SenSi-1 Agrinak chicken)

(Org: Eng)

JITV 22(2): 68-79

One of local chicken breeds develop in Indonesian Research Institute for Animal Production (IRIAP) is local SenSi-1 Agrinak chicken. This new improved local-meat-type breed was released with Ministry Agriculture Decree Number 39/Kpts/PK.020/1/2017 on 20th January 2017. SenSi-1 Agrinak was originally selected from native Sentul chicken breed obtained from Ciamis district in West Java Province. Selection criteria were two feather colors of grey or black spotted white (pucak), which were applied to both males and females. Pea-comb type was also one criterion for males, applied at the age of 10 weeks. Live weight at the age of 10 weeks with the selection intensity of 25% was applied to each generation of males chicken. Selection proceeded for six generations. Selection program was carried out under standard feed formulae containing around 17% crude protein with 2850 kcal ME/ kg, and containing other nutrients following the ones recommended for modern chicken of White Leghorn. Observation was conducted on each of about 2000 young chickens of grey and of Pucak SenSi-1 Agrinak both males and females age of 10 and 84 weeks. Results showed that grey SenSi-1 Agrinak chicken in total population, had: i) Grey feather color distribution of 55.51% in males, and 60.77% in females; ii) Yellow shank color of 52.51% in males, and 33.33% in females; iii) Pea type comb of 90.98% in males, and 89.23% in females; iv). Ten weeks live weight of 886.38+142.93 g/bird in males, and 739.17+ 118.87 in females. Pucak SenSi-1 Agrinak chicken in total population, had: i) Pucak feather color of 75.65% in males, and 8330% in females; ii) Yellow shank color of 51.91% in males, and 36.59% in females; iii) Pea type comb of 91.55% in males, and 92.28% in females; iv) Ten weeks live weight of 908.76+ 130.98 g/bird in males, and 750.53+ 110.56 g/bird in females. Whilst for grey SenSi-1 Agrinak male chicken after selection had live weight at 10 weeks old of 1015+107 g/bird, and for Pucak SenSi-1 Agrinak male chicken was 1051+76 g/bird. This initial performance information for those two breeds of considerably improved local chicken can be used as the base of information for SenSi-1 Agrinak breed for male line of meat type of local chicken breeding.

(Author)

Key Words: Qualitative Character, Quantitative Character, SenSi-1 Agrinak Chicken

UDC: 599.735.51

Sumarningsih (IRCVS, Bogor)
Susanti (IRCVS, Bogor)
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Evaluasi pada LipL32 ELISA untuk deteksi bovine leptospirosis di Jawa Barat (Evaluation of LipL32 ELISA for detection of bovine leptospirosis in West Java)

(Org: Eng)

JITV 22(2): 80-90

The current diagnosis of leptospirosis, micro Agglutination Test (MAT) and isolation, is expensive, impractical and technically demanding. This study was aimed at developing an ELISA based on recombinant LipL32 as a practical, inexpensive test for Leptospirosis. The DNA encoding LipL32 was isolated from Leptospira pomona, inserted into pRSET-C plasmid then expressed in E.coli BL21 as a poly-histidine-tagged protein. The amount of LipL32 protein, which was purified from the supernatant of lysed cells by a Ni-NTA column, was 1mg/l culture. This purified LipL32 was used as the coating antigen at 5µg/ml. The accuracy of ELISA was evaluated based on ROC analysis, by comparing the ELISA and MAT results of 517 bovine sera. Result in this study showed that the area under curve (AUC) was 0.853, which categorised the LipL32 ELISA as a “moderately accurate” test and indicates that the ELISA was able to differentiate positive and negative Leptospirosis serum. The result also showed ELISA LipL32 could detect serum positive MAT to Hardjo, Grippotyphosa, Tarrasovi, Rachmati and Bataviae. The optimal cut off for OD ELISA determined based on ROC curve was 0.504, and it showed sensitivity and specificity of ELISA LipL32 relative to MAT were 86.0% and 69.5%, respectively. Overall, the result in this study showed that ELISA LipL32 can be used as a rapid test for identification of anti-Leptospira antibodies in bovine.

(Author)

Key Words: ELISA, LipL32, Leptospirosis, Bovine

UDC:

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Deteksi serotipe, karakterisasi molekuler dan studi kekerabatan genetik isolat lokal *Pasteurella multocide* (Serotype detection, molecular characterization and genetic relationship study on *Pasteurella multocide* local isolate)

(Org: Eng)

JITV 22(2): 91-99

Pasteurella multocide is a bacteria that causes snoring disease or Haemorrhagic Septicaemia (HS) in Indonesia with high mortality and morbidity in heterogeneous species including cattle as a source of animal products with high economic value. The complexity of conventional and biochemical identification is a major obstacle in the detection of this disease because P. multocide has five serotypes A, B, D, E and F, while serotype B is the leading cause of HS cases in Asia including Indonesia. Therefore, it is necessary to conduct a research that can determine the serotype and molecular characterization and genetic study of five isolates of *P. multocide* from Lampung and Kupang by Polymerase Chain Reaction (PCR) technique. After PCR was performed on specific genes, capsular genes, 16S rRNA genes, sequencing and analysis using Bioedit, BLASTn, CLUSTALW and MEGA7.0.25, it was found that the five isolates were divided into two serotype groups: A and B. Isolate *P. multocide* (code: PMc) from Lampung is high homolog with ATCC isolate 12945, so it can be used as a positive control serotype A in the detection of other P. multocide isolates with PCR. Whereas, isolate *P. multocide* from Kupang can be used as positive control of serotype B because it is identical to *P. multocide* PMTB2.1 (CP007205.2) from Malaysia that is isolated from buffalo infected by HS.

(Author)

Key Words: *Pasteurella multocide*, Snoring Disease, Detection, Polymerase Chain Reaction (PCR), Sequencing

UDC: 633.3

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Peningkatan nilai gizi kulit buah kakao melalui fermentasi oleh *Aspergillus* spp. dengan dua tingkat urea dan amonium sulfat (Improvement of nutritional value of cocoa pod husk fermented by *Aspergillus* Spp. with two levels of urea and ammonium sulphate)

(org: Eng)

JITV 22(3): 101-113

Cocoa pod husk is abundant as a waste product of cocoa plantation and potential as feed ingredient but has low nutritional value. To increase the nutritive value of cocoa pod husk (CPH), biological process through solid substrate fermentation using *Aspergillus oryzae* and *Aspergillus niger* and addition of two doses (N1 and N2) of nitrogen mixture had been done. The product is Fermented Cocoa Pod Husk (FCPH). Protein content increased from 50 g/kg before fermentation to 133.8 g. kg^{-1} for N1 for *A. niger* and 150 g/kg using *A. oryzae*. True protein were 99.8 and 93.5 g/kg for N1 and N2 treatments (*A. niger*); 119 and 104.1 g/kg for N1 and N2 treatments (*A. oryzae*). *Aspergillus niger* showed a superiority in term of enzymes production when compared to *Aspergillus oryzae*. Mannanase activy in *A. niger* fermentation product with N1 dose reached up to 2654 U/g and *A.oryzae* was 1122 U/g. Dry matter and protein digestibility for *A. niger* FCPH were 47 and 57% and *A. oryzae* FCPH were 52 and 62% repectively. Fermentation processed of CPH yield a product that very potential as an alternative feed with higher in protein content and contain mannanase enzyme.

(Author)

Key Words: Cocoa Pod Husk, *Aspergillus niger*, *Aspergillus oryzae,* Mannanase, Cellulose

UDC: 633.31/.37

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Identifikasi kualitas fisik dan pelet konsentrat hijau berbasis *Indigofera zollingeriana* secara *In Vitro* (The physical quality and digestibility in vitro determination of green pellet concentrate based on *Indigofera zollingeriana*)

(Org: Eng)

JITV 22(3): 114-123

This study aims to determine the physical quality and digestibility of green pellet concentrate based on *I. zollingeriana*. The composition of green pellet concentrate feed contains *I. zollingeriana* and *C. calothyrsus* in the following combinations: 1) *I. zollingeriana* /*C. calothyrsus* (90/0; R0), 2) *I. zollingeriana*/*C. calothyrsus* (75/15; R1), 3) *I. zollingeriana*/*C. calothyrsus* (60/30; R2) and 4*) I. zollingeriana* / *C. calothyrsus* (45/45; R3). The physical quality study of green pellet concentrate was performed using a completely randomized design with four types of green concentrate as treatments of three replications. Green pellet concentrate studies conducted in vitro rumen fermentation were performed using in a randomized block design with four treatments and three sources of liquid rumen as a block. The results of the experiments conducted with pellet criteria containing *I. zollingeriana/C. calothyrsus* composition (60/30; R2) have the best physical character (water activity = 0.58; particles size = 12.69 mm; degree value angles =

21.01°; collision endurance = 99.78%; endurance friction = 90.42 %; bulk density = 590 kg/m3) compared to the other treatments. The quality of diet based on digestibility in vitro, dry matter and organic material having a pattern equal to R0 is higher ($P<0.05$) compared with other treatments, while the rough digestibility of In Vitro protein is seen R0, R1, and R2 ($P>0.05$), but ($P<0.05$) was higher than the treatment of R3. There was no difference of pH and total bacteria on green pellet concentrate type. The treatment of R3 has the lowest NH3, VFA and total gas concentration ($P<0.5$) compared with R2, R1 and R0. It was concluded that concentrate green pellet of *I. zollingeriana* composition (60%) and *C.calothyrsus* (30%) had better physical and nutritional quality.

(Author)

Key Words: *I. zollingeriana, C. calothyrsius*, Green Concentrated Pellets, Feed

UDC: 633.31/.37

Hutasoit, R. (IGRS, Medan)

Evaluasi empat spesies legume herba sebagai sumber hijauan dan *cover crop* pada perkebunan kelapa sawit (Evaluation of four pasture legumes species as forage and cover crop in oil palm plantation)

(Org: Eng)

JITV 22(3): 124-134

Pasture legumes is a very high quality of forage. The limited land is the problem of its development. Integration with oil palm plantations is one of the potentials for its development. This study was aimed to investigate the productivity of several legumes (*Arachis glabrata, Stylosanthes guianensis, Clitoria ternatea*, and *Chamaecrista rotundifolia*) as forages and cover crop. The potential tests were conducted in oil palm area of 4608 m2, in a complete block design with four treatments (legume species) and three replications. Parameters observed were: Legum production, leaf/stem ratio, chemical composition of legume, concentration of N, P in the soil, microbes in the soil, leguminous digestibility and palm fruit production. Results showed that the highest legume production (DM) was ($P<0.05$) in the species of *Clitoria ternatea* (16.15 tons ha-1year-1), the highest leaf/stem ratio ($P<0.05$) was in the *Arachis glabrata* (2.09). The chemical composition (DM) did not differ ($P>0.05$) ranged from 33.75 to 35.75%, the organic matter (OM) varied greatly ($P<0.05$) the highets was in *Clitoria ternatea*. The highest Crude protein ($P<0.05$) was in *Clitoria ternatea* 17.84%. NDF concentrations did not differ ($P>0.05$). The lowest ADF concentration ($P<0.05$) was in *Chamaecrista rotundifolia*. The concentration of N in the soil indicated that early year of activity was similar (average 0.10%), at the end of activity increased ($P<0.05$) in treatment *Stylosanthes guianensis* (0.16%). The highest population of N-fixation bacteria of 1.76x109 and phosphate solvent of 9.8x105 were in the treatment of *Clitoria ternatea*. Production of fresh fruit bunches of the palm was relatively similar ($P>0.05$) ranged from 16.52-19.21 tons ha-1year-1. It is concluded that *Clitoria ternatea* is the best species of legume tested as forage and cover crop in oil palm plantations.

(Author)

Key Words: Legume, Forage, Oil Palm

UDC: 633.3

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Evaluasi performa beberapa kultivar Sorghum bicolor sebagai sumber hijauan pakan di lahan kering iklim kering (Evaluation on performance of some Sorghum bicolor cultivars as forage resources in the dry land with dry climate)

(Org: Eng)

JITV 22(3): 135-143

The aim of this study was to evaluate the performance of several Sorghum bicolor cultivars as forage on a dry land with pH of 5.4, N of 0.08%; C/N of 9%, P of 0.06% and K of 0.01%. Nine cultivars of S. bicolor (Super 1, Super 2, Numbu, Kawali, G2, G5, PAC 537, PAC 593 and PAC 501) were evaluated. Plot size was 16 m2 with space planting of 15 x 75 cm. The experimental design used was randomized block design with three replications. Parameters observed were plant height, time of flowering, forage production and quality. The result showed that the primary plant growth was not different in all cultivars. In the 65 days old primary plant, the Super 2, PAC 537 and Kawali had no flower yet. Biomass production varied in primary plant between cultivars of 11.35 - 26.17 kg/16 m2. The highest biomass production was obtained in PAC 537 of 26.17 kg/16 m2 (16.34 t/ha) which were significantly higher than G2 of 11.35 kg/16 m2 (7.09 ton/ha) and was not significantly different with other cultivars. In the 45 days ratoon I, Super 2, G5 and Super 1 showed faster growth. Biomass production increased in the ratoon I around 19.88 kg/16 m2 (12.42 ton/ha). PAC 537 produced the highest biomass of 30.14 kg/16 m2 (18.84 ton/ha) and was not significantly different with other cultivars, except with the G2. Biomass production of ratoon II decreased around 1.83 kg/16 m2 (1.14 t/ha)–4.77 kg/16 m2 (2.98 t/ha) and increased in the ratoon III of 15.72 kg/16 m2 (9.82 t/ha)–26.05 kg/16 m2 (16.28 t/ha). The quality of forage ratoon I was better compared to the primary plant with the lowest one was in ratoon II. It could be concluded that Super 1, Super 2 and PAC 537 cultivars might be recommended as potential forage.

(Author)

Key Words: Sorghum, Cultivar, Biomass Production, Quality

UDC: 591.465.3

Kostaman, T. (IRIAP, Bogor)
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Gambaran primordial germ cell yang diinkubasi selama 24 jam dalam larutan phosphat bufer saline [-] (Primordial germ cells profiles incubated for 24 hours in phosphate buffer saline [-] solution)

(Org: Eng)

JITV 22(3): 144-150

Gonadal development is a sequential process that can be divided into three major events: the PGCs migration, sex determination and gonadal differentiation. This study was aimed to see the development of PGCs isolated from the gonads of embryos after being incubated for 7 days and then was incubated using a solution of Phosphate Buffer Saline (PBS) [-]. The developing gonad can be isolated from 7 days old chick and can be incubated at a temperature of 37.8oC in a solution of PBS [-]: without Ca2+ and Mg2+. The release of gonadal PGC was observed within 1, 8, 16, and 24 hours after the embryonic gonad was placed in a PBS solution [-]. The results showed that PGCs can be separated from gonadal tissues and can be collected by entering the developing gonad to the PBS [-] solution. The highest percentage of PGCs and survival rate was obtained after gonad was incubated for 1 hour and was not different with 8 hours ($P>0.05$). Those result was significantly different ($P<0.05$) with the 16 and 24 hours incubation. The highest purity rate percentage was in the 8 hours incubation, but did not show a significant difference ($P>0.05$) with the 1 and 16 hours incubation. The percentage of the purity differed ($P<0.05$) after the 24 hours incubation. It can be concluded that the most appropriate incubation time to obtain PGCs from the KUB chicken embryonic gonad is no more than 8 hours.

(Author)

Key Words: Gonad, PGCs, Native Chicken

UDC: 578.81

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Profil sitokin tikus yang terinfeksi dengan virulensi tinggi dan rendah isolat *T. evansi* Indonesia (Cytokines profile of mice infected by high and low virulences of Indonesian *T. evansi* isolates)

(Org: Eng)

JITV 22(3): 151-164

Surra in livestock is caused by *Trypanosoma evansi*, a homoflagella blood protozoa that circulate in extracellular. This disease is widespread in Asia, Africa, South and Central America. According to the immunological aspect, the severity of surra in livestock and mice which infected by trypanosoma is associated with an inflammatory response. On the other hand, the survival time of mice depends on the regulation of Th1 synthesis and proinflammatory cytokines such as IFN-γ and TNF-α. The aim of this study was to observe the responses of proinflammatory cytokines IFN γ, TNF-α and anti-inflammatory IL-10 which result from interaction with parasites. This information is needed for improvements in the management of prevention of Surra in animals. A total of 30 mice were divided into 3 groups. The group was infected with a low virulency *T. evansi* (Pml287); high virulence (Bang87) respectively and one group was not infected as control. Mice sera were collected in every 4 days for cytokine measurement using an Enzyme Link-Immunosorbent Assay (ELISA). The result showed a difference response of proinflammatory and antiinflammation cytokine profile between the infected mice by isolates Bang 87 and Pml 287. Early deaths in mice infected by Bang 87 isolate were suspected as a result of the response of systemic inflammation syndromes characterized by elevated IFN-γ levels that were not adequately compensated by anti-inflammatory. Anemia contributes to the cause of death in mice that support multiple organ failures (multiple organ disfunction).

(Author)

Key Words: Surra, *Trypanosoma evansi*, Cytokine, IFN γ, TNF-α, IL-10

UDC: 636.2.033

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Polimerfisme genetik dari gen IGF1, GH dan OPN pada persilangan sapi PO berdasarkan tipe kelahiran di Jawa Tengah (Genetic polymorphisms of IGF1, GH, and OPN genes in crosses Peranakan Ongole cattle based on birth type in Central Java)

(Org: Eng)

JITV 22(4): 165-172

Genetic Polymorphisms of IGF1, GH, and OPN Genes in Crossbred of Peranakan Ongole Cattle Based on Birth Type in Central Java. Improved productivity by crossing Peranakan Ongole (PO) cattle to exotic beef cattle through AI mating is preferred by many farmers in Central Java. Fertility determines the ability of a cow to give birth. IGF1 gene is predicted to affect either twin or multiple births in cattle, whereas GH and OPN genes determine reproductive fertility. Genetic polymorphisms of these three genes were studied in the crossbred of PO cattle for single birth (S) as well as twin and multiple (M) births or their historical (M) ones from two districts in Central Java, from Sragen (S = 7 hd and M = 13 hd.) and from Kendal (S = 9 hd, and M = 16 hd.). Genetic polymorphisms were identified by PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) method using restriction enzymes of *Sna*BI (IGF1 gene), *MSp*I (GH gene), and *Bsr*I (OPN gene). IGF1|*Sna*BI locus in the observed crossbred PO did not have SNP polymorphism or monomorphic for single and twins or multiple births. In contrast, GH|*MSp*I and OPN|*Bsr*I loci were polymorphic with quite a high degree of diversity. In conclusion, the IGF1|*Sna*BI locus cannot be used to investigate possible genetic control of twins and multiple births, whilst GH|*MSp*I and OPN|*Bsr*I polymorphisms can be considered as initial information for selection on fertility traits in beef cattle.

(Author)

Key Words: Beef Cattle, Genetic Polymorphism, Fertility Genes

UDC: 636.2.033

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Pengaruh status ovarium sapi Bali terhadap maturasi inti oosit dan tingkat fertilitas *in vitro* (Effect of Bali cattle ovarian status on oocytes nuclear maturation and *in vitro* fertilization rate)

(Org: Eng)

JITV 22(4): 173-178

The aim of this study was to investigate whether the reproductive status influences the nuclear maturation and fertilization rates of bali cattle oocytes in vitro. Several pairs of ovary were classified into four groups: 1) ovaries with Corpus Luteum (CL) and Dominant Follicle (DF), 2) ovaries without CL and with DF, 3) ovaries with CL and without DF, 4) ovaries without both CL and DF. In the first experiment, oocytes were collected by slicing method in Phosphate Buffer Saline (PBS) medium supplemented with 10% Fetal Bovine Serum (FBS) and 100 IU/ml penicillin streptomycin. Oocytes were matured in tissue culture medium (TCM)-199 supplemented with 10% Fetal Bovine Serum (FBS), 10 IU/ml Follicle Stimulating Hormone (FSH), 10 IU/ml Luteinizing Hormone (LH), and 50 µg/ml gentamycin. Oocytes were matured in 5% CO2 incubator, 38oC for 24 h. In the second experiment, oocytes were matured and then fertilized in vitro to observe pronuclear formation. The first experiment showed that the percentage of oocytes reached methaphase-II (MII) stage on ovaries with CL and without DF (89.47%) were higher (P<0,01) compared to ovaries without both CL and DF (75,47%), ovaries without CL and with DF (74.,41%), or ovaries with CL and DF (65,52%). The result of second experiment showed that the ovarian reproductive status was not significantly different (P>0.05) on fertilization rate.

(Author)

Key Words: Ovarian Status, Oocytes, Bali Cattle, Maturation, Fertilization

UDC: 633.255

Puastuti, W. (IRIAP, Bogor)
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Suplementasi molases dan asam amino bercabang pada tongkol jagung amoniasi sebagai pakan ruminansia untuk meningkatkan kecernaan *in vitro* (Supplementation of molasses and branched-chain amino acid to increase *in vitro* digestibility of ammoniated corn cob in ruminants feed)

(Org: Eng)

JITV 22(4): 179-187

Corn cob contains high fiber and lignin which causes low nutritive value. The objective of the study was to improve the digestibility of ammoniated corn cob (CC) by supplementation of molasses and branched-chain amino acid (BCAA: valine, leucine and isoleucine). CC was processed by addition 3% urea. The first stage of in vitro test was done with 4 levels of molases 0, 5, 10 and 15% of dry matter (DM) of CC. The experiment was carried out using complete randomized design with 4 treatments and 4 replications. The second stage was also in vitro study of supplementation of two levels each for valine = V, leucine = L and isoleucine = I namely 0.1 and 0.2% of DM of ammoniated CC. There were 8 treatments combination of the BCAA as follow: A = V0.1 L0.1 I0.1; E = V0.2 L0.1 I0.1; B = V0.1 L0.1 I0.2; F = V0.2 L0.1 I0.2; C = V0.1 L0.2 I0.1; G = V0.2 L0.2 I0.1; D = V0.1 L0.2 I0.2; H = V0.2 L0.2 I0.2 and 1 control (V0.0 L0.0 I0.0). The experiments were done using completely randomized design with 9 treatments and 3 replications. The results showed that treated CC with urea was able to increase protein content by 78% (increased from 3.34% to 5.95%) while neutral detergent fiber (NDF) decreased by 15.4%, acid detergent fiber (ADF) by 7.9% and lignin 16.7%. Addition of molasses in ammoniated CC increased DM digestibility (P <0.05) by 7.5% (41.9 vs 43.51-46.26%) and NDF by 17.7% (38.41 vs 43.76 – 46.31%). Supplementation of BCAA resulted in the highest DM, OM and NDF digestibility (P <0.05) in the treatment of A, C, D and G. Compare to treament I, the digestibility of DM, OM and NDF in G treatment increased by 31.4%, 27.5% and 36.5%, respectively and produced the highest total population of rumen bacterial of 12.4 x 109 colonies /ml. It can be concluded that the digestibility of ammoniated CC increased by the supplementation of 5% molasses and BCAA combination consisted of 0.2% valine, 0.2% leucine and 0.1% isoleucine.

(Author)

Key Words: Corn Cob, Ammoniation, Molasses, Branched-Chain Amino Acid

UDC: 636.39

Ginting, S.P. (IGRS, Medan)
Simanihuruk, K. (IGRS, Medan)
Antonius (IGRS, Medan)
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Pertumbuhan dan pemanfaatan pakan kambing persilangan Boer x Kacang yang diberi pakan komplit dengan kandungan protein dan energi berbeda (Growth and feed utilization of Boer x Kacang crossbred goats offered total mixed rations of different protein and energy levels)

(Org: Eng)

JITV 22(4): 188-195

The aim of this study was to evaluate the growth of and feed utilization by Boer x Kacang crosses goats fed on total mixed ration differing in protein and energy levels. Four total mixed rations combination were formulated to contain 16 and 18% crude protein and 2650 and 2850 Kcal ME/ kg DM (dried matter) energy density. Twenty-eight male Boer x Kacang crosses goats (14.5 ± 1.14 kg) and of age ranging from 4 to 5 months were randomly allocated to one of these four TMRs (total mixed rations) (7 animals/TMR). Animals were put in individual metabolic cage equipped with troughs and water container. The effects of dietary treatment were assessed using the general linear model and significance of the diet effects was detected using Duncan's multiple range test. Dry matter intake increased as metabolizable energy density of diet increased from 2650 to 2850 Kcal/kg DM, but it is not affected by increasing crude protein level from 16 to 18%. The average daily gains were not improved ($P>0.05$) as the crude protein levels and metabolizable energy density of diet increased. Crude protein levels and ME density did not affect ($P>0.05$) the DM, OM and energy digestibility, but NDF digestibility was affected by the ME density of diets ($P<0.05$). Daily N intakes were greater ($P<0.0\%$) in goats received diets higher in the crude protein and metabolizable energy levels. Fecal N output was not influenced ($P>0.05$) by the crude protein and metabolizable energy levels, but urinary N output was higher ($P<0.05$) as the level of diet crude protein increased. The N retention was affected ($P<0.05$) by the crude protein levels but not ($P>0.05$) by the metabolizable energy density of diets. The BUN (blood urea nitrogen) was not affected by the metabolizable energy density but it was higher ($P<0.05$) in goats offered diets with higher crude protein contents. The plasma glucose level was affected by the metabolizable energy density ($P<0.05$), but not by the crude protein levels. It is concluded from the present study that the Boer X Kacang crossbred goats have high level of DM intake when offered feeds in the form of total mixed rations. At this high rate of feed intake this type of goats are able to gain optimally when offered feed with crude protein level of 16% and metabolizable energy density of 2850 Kcal/kg DM.

(Author)

Key Words: Goats, Total Mixed Ration, Protein level, Energy Level

UDC: 636.58.033

Wiedosari, E. (IRCVS, Bogor)
Wardhana, A.H. (IRCVS, Bogor)

Aktivitas antikoksidia artemisinin dan ekstrak daun *Artemesia annua* pada ayam yang diinfeksi dengan *Eimeria tenella* (Anticoccidial activity of Artemisinin and Extract of *Artemesia annua* leaves in chicken infected by *Eimeria tenella*)

(Org: Eng)

JITV 22(4): 196-204

The continuous use of anticoccidial drug in chicken often continuously generates drug resistance and tissue residue; so thatconsequently, a safe alternative anticoccidial drug based on herb is fundamentally required. The aim of thise study was to examine anticcocidial activity of artemisinin and extract of *Artemesia annua* leaves in chicken infected by *Eimeria tenella*. A total of 35 chickens of Cobb strain was divided into seven groups with five replicates birds per group, i.e. uninfected chicken group (P I), infected but untreated chicken group (P II), infected and treated chicken group with 8.5 ppm, 17 ppm, 34 ppm, for P III, P IV and P V respectively, infected and treated chicken with 17 ppm of *A. annua* extract (P VI) and infected and treated chicken with Sulfa (P VII). All chicken, except the uninfected group, whereas infected with 2000 infective oocyst of *E. tenella* except the uninfected group. Treatment was delivered by oral, once per day for eight days. The criteria observed were clinical manifestation of chickens, number of oocyst in feces, body weight, cecal lesion score, haematocrit (packed cell volume) and haemoglobin value. The results showed that extract of *A. annua* leaves (P VI) was the most effective treatment to reduce the number of oocyst in feces (74.18%), followed by 34 ppm of artemisinin group (P VII). In addition, application of *A. annua* extract and artemisinin was significantly able to decreased the cecal lesion score ($P<0.05$). Even though body weight and Hb value were not indifferent significantly different ($p>0.05$), however *A. annua* extract and artemisinin treatments were significantly able to hold PCV value on normal level compared to P II and P IV ($P<0.05$). It concluded that extract *A. annua* leaves and artemisinin could be used an alternative anticoccidial in chickens.

(Author)

Key Words: *Eimeria tenella*, Artemisinin, *Artemesia annua*, Coccidiosis

UDC: 636.2.034

Rachmawati, F. (IRCVS, Bogor)
Ariyanti, T. (IRCVS, Bogor)

Kontaminasi bakteri *Escherichia coli* O157:H7 pada peternakan sapi perah (Contamination of *Escherichia coli* O157:H7 in dairy cow farms)

(Org: Eng)

JITV 22(4): 205-211

E. coli serotype O157:H7 is a foodborne pathogen that endangers human health. Cattle are the main reservoir of this bacterium. *E. coli* O157:H7 that come out of the livestock can contaminate the surrounding environment, dairy products, meat, vegetable or fruit crops, so it can act as a source of infection and disease in humans. This research was conducted to identify the contamination of *E. coli* O157:H7 on dairy cow farms so the current situation is known. Sampling area for this research was dairy cow farm in Depok, Cianjur, Sukabumi, and Bandung. Types of samples taken are cow anal swab or fresh feces and livestock drinking water. Isolation of *E. coli* bacteria was done on mTSB media, agar MacConkey, and Eosin Methylene Agar. Identification of bacteria as *E. coli* O157:H7 was done by biochemical test and for serotype determination was used agglutination test using monospesifik antiserum for O157 and H7. The results of research showed that of the total 178 samples had 126

(70.8%) samples containing *E. coli* bacteria. Of the 126 samples containing *E. coli* bacteria, 94 (74.6%) were determined as *E. coli* serotype O157:H7. The presence of *E. coli* O157:H7 contaminants on dairy cow farms, their potential impacts on human health, prevention and control of the disease is discussed in this paper.

(Author)

Key Words: Dairy Cow, *E. coli*, *E. coli* O157:H7, Environment

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   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.
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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.

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Patent:

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

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