PROCEEDING
INTERNATIONAL CONFERENCE
6th SAADC 2017

Conference on Sustainable Animal Agriculture for Developing Countries

"WISDOM OF USING LOCAL RESOURCES FOR DEVELOPMENT OF SUSTAINABLE ANIMAL PRODUCTION IN DEVELOPING COUNTRIES"

The Singhasari Resort, Batu City, Indonesia, October 16-19, 2017

Jointly organized by

Faculty of Animal Husbandry
Brawijaya University Indonesia

Institute of Tropical Agriculture
and Food Security

ANIMAL PRODUCTION SCIENCE

Australian Aid

ISBN 978-974-625-711-4

PROCEEDING

The 6th International Conference on Sustainable Animal Agriculture for Developing Countries (SAADC 2017)

October 16-19, 2017
The Singhasari Resort, Batu City, Indonesia

Organized by:

Faculty of Animal Husbandry, Brawijaya University, Indonesia

In Collaboration with:

Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Malaysia

Australia's Aid Program, Department of Foreign Affairs and Trade, Australian Government

Animal Production Science Journal, The Commonwealth Scientific and Industrial Research Organization (CSIRO)

Published by
Faculty of Animal Husbandry, Brawijaya University
Jl. Veteran, Malang 65145, East Java, Indonesia
Homepage http://www.fapet.ub.ac.id E-mail : fapet@ub.ac.id
Telp. (0341) 553513, 551611 Pes. 211, Fax. (0341) 584727
ISBN 978-974-625-711-4

PREFACE

It is my privilege to thanks to all of authors for your enthusiasm in participating and contributing papers at this 6th International Conference on Sustainable Animal Agriculture for Developing Countries (The 6th SAADC-2017) that had been successfully held on 16-19 October 2017 in The Singhasari Resort, Batu City, Indonesia with the theme of *"Wisdom of Using Local Resources for Development of Sustainable Animal Production in Developing Countries"*

The primary objective of the 6th SAADC-2017 was to provide a scientific forum for animal scientists and producers, and administrators of livestock related agencies, particularly from the developing countries, to share their experiences, discuss issues and suggest recommendations to develop further a more sustainable livestock production.

This proceeding contains selected papers that were presented in the conference based on the quality and relevancy to the confencence. The papers are reflecting responsiveness of animal scientist from various countries in promoting sustainability of animal agriculture for the prosperity of the never ending generations. These proceeding hopefully will certainly enrich the body of knowledge and understanding about various aspects related to sustainable animal agriculture.

Our special thanks are also for the SAADC President for his confidence to our Universitas Brawijaya to organize this prestigious conference. Also, congratulation that SAADC is now listed in the International Congress and Conference Association (ICCA) based on its quality and consistent activities.

We also wish to thank all partners and sponsors for their support to the success of the conference. To colleague members of the organizing committee, please accept my deep appreciation for your hard working in ensuring the success of the conference.

Yours Sincerely,

Prof. Ifar Subagiyo
Chief Editor

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Antibacterial and antifungal activities of *Muntinga calabura* leaves extract as alternative to antibiotic in mastitis treatment

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Abstract

Mastitis is the most damaging worldwide disease of dairy industry. The aim of the study was to evaluate the antibacterial and antifungal effects of *Muntinga calabura* leaves extract against mastitis infection in dairy cattle. Experimental treatments were subclinical mastitis milk added with different levels of ethanol extract of *Muntinga calabura* leaves (EEMC) (6.25%, 12.5%, 25% and 50%) in a completely randomized design with five replications. The result showed that total bacterial *Staphylococcus aureus* and fungal counts were significantly decreased ($P<0.01$) with increasing EEMC concentration. The EEMC concentration of 50% had the highest ($P<0.01$) antimicrobial activity among the groups. The EEMC could reduce 87.2% – 94.8%, of total bacteria, 71.1- 95.8% of *Staphylococcus aureus*, and 64.4% - 97.8% of fungi in mastitis milk. Taken together, current findings suggested that *Muntinga calabura* leaves have antibacterial and antifungal activities and can be used as an alternative to synthetic antibiotic for mastitis treatment to prevent the antibiotic residue in milk.

Keywords: mastitis, antibacterial, antifungal, Muntinga calabura, antibiotic alternative

Introduction

Mastitis is an inflammation of the mammary gland caused by bacterial infection. In the previous reserach, it has been reported that fungi is another causative agent of mastitis (Pachauri et al., 203). One of the challenge to treatments of bovine mastitis due to many of the causative agents, especially fungi do not respond to antibiotic therapy, however they use some antibiotics like tetracycline as their source of energy (Tarfarosh and Purohit, 2008). Moreover, the use of antibiotics to treatment the disease is limited due to public concerns for the antibiotic residue on milk and it will be resisten to the humans who consumed milk. Recently, numbers of studies focused to find alternatives to changes antibiotics with herbal plants. Plant-derived natural bioactive compound is very potential to be use as an alternative antibiotic for mastitis treatment. *Muntinga calabura* is one of the most common trees in Indonesia and have been used as a traditional medicine to treatment of fever, cold, liver, and to decrease gastritic. It also has anti-inflammatory activities (Balan et al., 2015). Therefore, this study was aimed to elucidate the potential of *Muntinga calabura* leaves extract as antimicrobial against bacteria and fungi in mastitis milk, as well as to evaluate the prevalence of mastitis in Central Java.

Methodology

Examination of mastitis prevalence was conducted in Semarang Regency, the center of dairy farming in Central Java. A total of 105 lactating cattle were tested by California Mastitis Test (CMT) (Bovivet, Kruuse Denmark). Milk samples from 20 quarters were aseptically collected and tested against *Muntinga calabura* leaves extract.

Muntinga calabura leaves were collected from the area of Diponegoro University in Semarang. Fresh leaves were oven-dried at 50°C for 24 h then grinded. The leaves powder was soaked in the 96% ethanol in the ratio of 1:10 (w/v) for 24 h. The supernatant was filtered and then evaporated using rotary evaporator at 40°C. The EEMC was dissolve in 10% DMSO just before used. The experimental groups were: mastitis milk added with either 6.25%, 12,5%, 25%, or 50% EEMC (M1, M2, M3, and M4 respectively), mastitis milk added with synthetic antibiotic (K+) and mastitis milk only (K-). The synthetic antibiotic was used TERREXINE (combination of Kanamycin and Cefalexine, Univet Ireland). Mastitis milk sample were analyzed for total bacterial count, gram staining, *Staphylococcus aureus* and fungal counts.

Results and Discussion

The positive results of CMT were categorized as clinical and sub-clinical mastitis. Mastitis prevalence was 67%, whereas the healthy udder was only 33% (Figure 1). In the current study, mastitis prevalence in Central Java was similar with in West Java, that is 67.5% (Susanti et al., 2017). The dominant cases were sub-clinical mastitis not sign of abnormality of mammary gland and milk, thus farmers could not recognize the symptoms and they were depend on the paramedic person to check the animal health status. Good milking practices may give contribution to the mastitis problem. Most of farmers applied traditional dairy practices. Our previous study reported that 80% of farmers used plastic bucket for milking rather than using stainless steel (Prihutomo et al., 2015) with improper disinfection procedure.

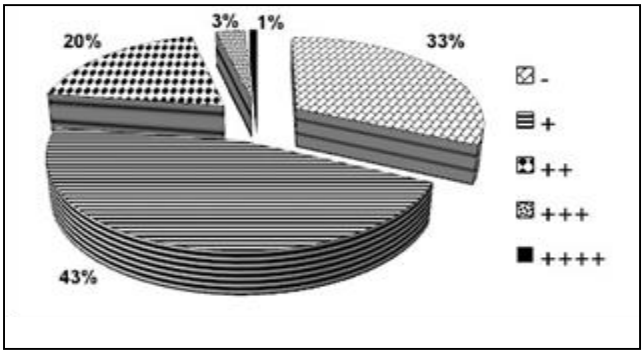


Figure 1. CMT result from each quarter

The ECMC at the concentration of 50% showed to possess the highest antibacterial and antifungal activity among the treatment groups (Table 1). The antimicrobial activity of synthetic antibiotic (K+) has shown the best among others, however the possibility of antibiotic residue in milk should be considered. Bacterial and fungal counts were significantly decreased ($P<0.01$) with increasing extract concentration. The number of total bacteria reduction in milk was varied from 87.2% to 91.4%, whereas the fungi reduction in milk was varied from 64.4% to 97.8% (Table 2). Since this is a preliminary screening for the presence of antimicrobial properties in the *Muntinga calabura* leaves extract, at the moment, the identification of chemical constituents or bioactive compounds is not part of the objective from this study. Based on previous studies on the antibacterial activities of *Muntinga calabura* leaves, the antimicrobial activity is suggested to be due to the presence of phytochemical compound such as sterol, flavonoid, alkaloid, saponin, glycoside and tannin (Buhian et al., 2016). Gram staining of the individual colony has showed gram-positive cocci and gram-negative bacilli spore-forming bacteria (Figure 2). The mechanisms by which microorganism survive the action of antimicrobial agents still poorly understood. However, the present data suggest that ethanol extract of *Muntinga calabura* leaves is very potential used as an alternative to synthetic antibiotic due to high antibacterial and antifungal activity.

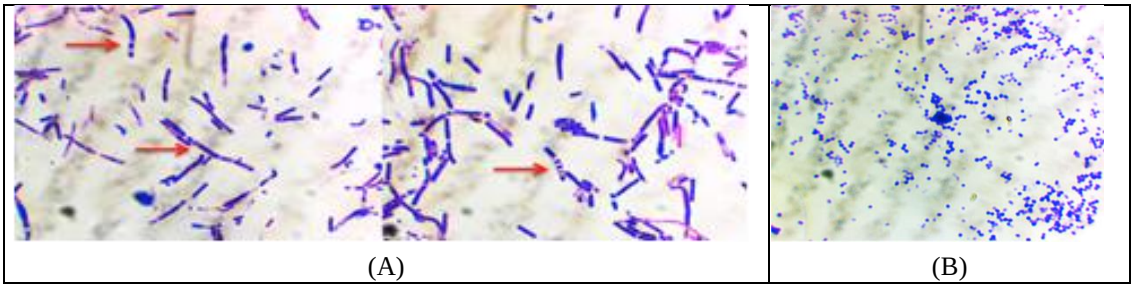
Table 1. The number of total bacteria, *Staphylococcus aureus* and fungi in mastitis milk added with ethanol extract of *Muntinga calabura* leaves (EEMC)

Microbes (cfu/ml)	K-	M1	M2	M3	M4	K+	National standard
Total bacteria	$3.711.299^{A}$	472.800^{B}	395.200^{B}	191.200^{C}	131.200^{C}	0^{D}	Max 10^{6}
S. aureus	18.850^{A}	5.450^{B}	2.750^{B}	2.100^{C}	800^{D}	0^{D}	Max 100
Fungi *	450^{A}	160^{B}	110^{B}	40^{C}	10^{C}	0^{D}	-

*The maximum tolerated amount of fungi contamination in milk is not available at the Indonesian National Milk Quality Standard SNI 3141.1:2011. Fungi is defined as yeast and mold; ^{ABCD}Different superscript letters in the same row denotes significance (P<0.01)

Table 2. Percent decrease in the number of bacteria and fungi in milk

Parameters	M1	M2	M3	M4
Total bacteria (%)	87.2	89.4	91.4	94.8
Staphylococcus aureus (%)	71.1	85.4	88.9	95.8
Fungi (%)	64.4	75.6	91.11	97.8



(A) (B)

Figure 2. Gram-negative bacilli spore-forming bacteria (red arrow) (A), and Gram-positive cocci bacteria (B)

Conclusion

A *Muntinga calabura leaf* has antibacterial and antifungal activities and can be use as an alternative to synthetic antibiotic for mastitis treatment to prevent the antibiotic residue on milk.

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Factors influencing the incidence of *Eimeria leuckarti* in horses

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Abstract

A cross sectional survey was carried out to determine the prevalence of coccidiosis in horses and risk factors associated with their prevalence in district Toba Tek Singh, Punjab from April, 2009 to March 2010. Faecal samples were collected from whole the district using two stage cluster random sampling method and analysed by standard parasitological procedures. Of the total 484 faecal samples examined for *Eimeria*, 244 (50.41%) were found infected with *Eimeria leuckarti*. Peak prevalence was observed in August (OR=1.156; χ^2=20.055) indicating higher prevalence at higher humidity while least number of animals were found infected with *Eimeria leuckarti* in months of April to June, being the driest period of the year in Pakistan. Wet season was found favourable for propagation of *Eimeria*. Foals (124/197; 62.94%; OR=0.422; χ^2=20.825) and mares (196/347; 56.48%; OR=0.512; χ^2=13.265) had significantly higher prevalence (P<0.05) of *Eimeria* than adults (120/287; 41.81%) and males (48/137; 35.04%) respectively. Among management and husbandry practices; Farming type, feeding system and floor type strongly influenced the prevalence of *Eimeria*. Coccidosis was more prevalent in mix farming, ground fed, pond watered animals and non-cemented floor (P<0.05) as compared to single farming, tap watered animals, trough fed and partially cemented floor type respectively. Body condition of animals were not found risk factors (P>0.05) influencing prevalence of *Eimeria*. Study reports first time in detail the risk factors influencing prevalence of Eimeria in horses. Furthermore, this is first report of occurrence of Eimeria in horses of Pakistan.

Keywords: Eimeria, horse, prevalence, risk factors, Pakistan

Introduction

Coccidiosis in birds and domestic animals is caused by genus *Eimeria*. Over 1000 species of Eimeria have been identified. Most species of *Eimeria* are host specific. In horses, coccidiosis is caused by E. *leuckarti*, E. solipedum, and E. uniungulsti (Ghahfarrokhi et al., 2014). It is an enteric intracellular protozoa which belongs to phylum Apicomplexa. *Eimeria leuckarti* infect intestinal epithelial cells which then migrate to lamina propria. The disease is often associated with nonspecific digestive disorders viz poor assimilation of the nutrients and fermentation of the intestinal contents, loose irregularly formed bowels (Sudan et al., 2013).

Coccidiosis in domestic animals has been reported from various regions of Pakistan (Khan et al., 2011) but the information on Eimeriosis in horses in Pakistan is lacking. Moreover, research has been done on coccidiosis in birds and ruminants but little is known about life cycle, morphology, epidemiology and treatment of coccidiosis in horses. Because of emergence of drug resistance, research in parasites has been tuned for exploration of non-chemical control strategies. Knowledge about factors influencing the prevalence of Eimeria in one area or in a certain type of equine population assists in their diagnosis and effective control. Epidemiological investigations of risk factors are expected to leverage non-chemotherapeutic management options and to enhance more targeted use of drugs.

Methodology

A total of four hundred and eighty four animals were examined in this survey. Samples were collected from district Toba Tek Singh which is situated in Punjab Province of Pakistan. Two stage cluster random sampling technique was adopted for collection of samples. Primary units were union councils and elementary units were animals. Number of primary and elementary units was calculated by using formulae as described by Thrusfield (2008). Information regarding season, age, sex feeding system, floor type, farming system were collected on a pre designed questionnaire.

Five to ten grams of fresh fecal samples were collected in plastic bottles and preserved with 2.5% potassium dichromate. Fecal samples were examined microscopically through fecal flotation using saturated zinc sulfate solution. The procedure was adopted as described by Zajac and Conboy (2006). Quantitative fecal examination was performed by McMaster technique to determine the number of oocysts per gram of feces (OPG) as per the procedures of MAFF (1986). Identification of oocyst was carried out by determining morphological feature of oocyst like color, shape, size, presence or absence of micropyle and polar cap (Soulsby, 2006). Data about temperature, rainfall and relative humidity was recorded from meteorological cell, Department of Crop Physiology, University of Agriculture, Faisalabad.

Logistic analysis was carried out by using logit model including all variables in the model with backward elimination procedure. Factors with paired characteristics were analyzed using Odds Ratio (OR) and Mantel–Haenszel (M. H.) chi-square. Hosmer-Lemeshow goodness-of-fit test indicated that model fits well. All the analyses were carried out using SAS software package (1998) at 95% confidence level (SAS1998).

Results and Discussion

Only species identified in feces of horses of Toba Tek Singh was *Eimeria leuckarti*. Fecal examination of total number of samples (484) revealed that 50.41% (244) animals were found infected with coccidiosis. Analysis of all the hypothesized risk factors by stepwise multivariate logistic regression model and M. H. chi-square analysis revealed that season, age, sex, floor, farming and feeding system were the factors significantly influencing the prevalence of Eimeria in horse population of district Toba Tek Singh, Pakistan. Highest prevalence (74.36%) was found in month of August (rainy season). There was a strong negative correlation between age and occurance of infection. Odd's of finding oocycsts of Eimeria in foals during fecal examination was higher (62.94%; χ^2=20.8252) as compared to those in adults (41.81%; OR= 0.422). Females were found to be more frequently infected with coccidiosis (56.48%; χ^2=13.2653; OR=0.512) as compared to males (35.04%). Body condition of animals was found to be non-significantly associated (P value=0.2434) with infection.

Among management risks, farming type, floor type and feeding system were found to be significantly influencing the infection. Statistical analyses showed that Odd's of finding Eimeria was higher in feces of animals which are kept singly (58.06%; χ^2=11.8863; OR=2.671) as compared to those kept with other species like ovine, bovines, backyard poultry (42.37%). Higher prevalence was recorded in ground fed animals (62.17%; χ^2=9.5973; OR=1.741) as compared to trough fed animals (42.61%). Floor type strongly predisposed the infection in horses. Horses kept at non-cemented floor type was found to highly infected (58.04%, χ^2=12.5122, OR=) as compared to those kept at partially cemented / cemented floor type (41.92%).

This is the first large scale epidemiological survey investigating the association of various risk factors with the occurrence of Eimeria in horses. *Eimeria leuckarti* has been reported from various countries and its prevalence ranges 0.34% to 80% (Germany 64.9% &

80.0%, Greece 3.1%& 4%, Kashmir 0.34%, Nigeria 1.2%, Poland 6.7%, Romania 1.90%, Turkey 4.5%&5.88%, USA 41.0%) (Ghahfarrokhi et al., 2014; Papazahariadou et al., 2009). Higher prevalence may be due to unhygienic conditions, illiteracy, no regular deworming treatment.

It is believed that coccidiosis in horses mainly concerns the young horses especially foals. During an investigation on *Eimeria leuckarti* in horses of Romania, Ioniğă et al., (2013) observed higher prevalence in foals (i.e., 15%) comparing with the overall prevalence (1.90%). Papazahariadou et al. (2009) investigated occurance of Eimeria in four different age groups and found that 73% of 2 to 5 year-old age group, 45% of 6 to 10-year-old animals, 28% of 11 to 15 year-old animals, 14% of 16- to 20-year-old animals were infected. Similar results were recorded by Ghahfarrokhi et al., (2014). Higher prevalence in females has been recorded earlier in case of other species of Eimeria in domestic animals (Khan et al., 2011).

Results of our study support the principle that management systems are of major importance as predisposing factor to parasites. Lower infection in mix farming may be attributed to the fact that range of host species diluted the parasite concentration and hence lowered the prevalence as Eimeria species are host specific. Our findings of higher prevalence of Eimeria in ground fed horses are in agreement of recommendations of Radostits et al., (2009) to avoid the feeding on the ground as this increase the chances of contamination of the feed with Eimeria oocysts. Similar results were obtained by Papazahariadou et al. (2009) investigated that grazing horses were infected with more parasitic genera as compared to stabled horses. Mitchell et la. (2012) found that frequent emptying and cleaning of water troughs resulted in reduced exposure of infection and hence lower prevalence of Eimeria in cattle. The observation regarding higher prevalence in animals kept at non-cemented floor may be true because it is easy to clean cemented floor than non-cemented floor.

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