



PROCEEDING

International Seminar and 2nd Congress of SEAVSA

INCREASING ANIMAL PRODUCTION
THROUGH ZONOSSES AND REPRODUCTIVE DISORDER
HANDLING, AND THE IMPLEMENTATION
OF BIOTECHNOLOGY



Surabaya, 21-22 June 2011
Royal Ballroom, JW Marriott Hotel
Surabaya - Indonesia

Editors:

Mustofa Helmi Effendi (Indonesia)
Rahayu Ernawati (Indonesia)
Muhammad Yunus (Indonesia)
Jean-Francois CHARY (French)
Achariya Sailasuta (Thailand)
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Kristy Demmers (New Zealand)
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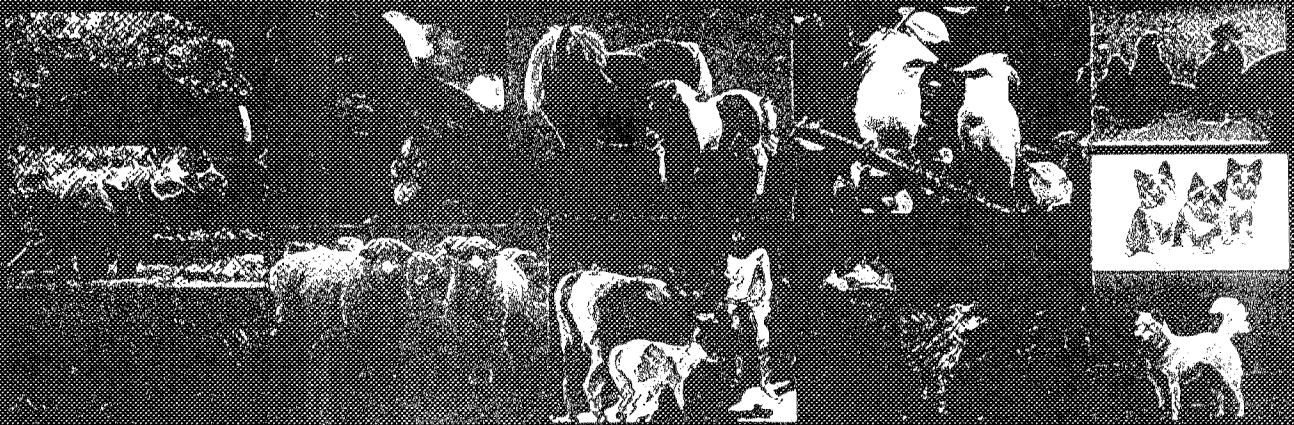
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Faculty of Veterinary Medicine Universitas Airlangga

I-MHERE Sub-component B.2.c Performance Based Contract



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AUP 600/45.426/12.11-B1E

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First print — 2011

Publisher:

Center Publishing and Printing of Airlangga University (AUP)

Kampus C Unair, Mulyorejo Surabaya 60115

Phone. +62 31 5992246, 5992247 Fax. +62 31 5992248

E-mail: aupsby@rad.net.id; aup.unair@gmail.com

Printed by: Center Publishing and Printing of Airlangga University (AUP)
(190/12.11/AUP-B1E)

Library of National Cataloging-in-Publication Data

Pro Proceeding International Seminar and 2nd Congress of SEAVSA:
Increasing Animal Production Through Zoonosis and Reproductive Disorder
Handling, and the Implementation of Biotechnology /
Ed: Mustofa Helmi Effendi ... [et al.] — First Print —
Surabaya: Center Publishing and Printing of Airlangga University, 2011
ix, 437 p.; 21 × 29,7 cm
Bibliography
ISBN 978-602-8967-51-8

1. Animals - Conservation Technology

I. Rahayu Ernawati
II. Muhammad Yunus

639.9

11 12 13 14 15 / 9 8 7 6 5 4 3 2 1

MEMBER OF IKAPI: 001 / JTI / 95

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INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) EXPRESSION IN THE BURSA OF FABRICIUS OF BROILERS INFECTED WITH VIRULENT GUMBORO VIRUS AND PROVIDES EXTRACTS *Annona squamosa* (Sweetsop)

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ABSTRACT

The bursa of fabricius serves as an important tissue in the process of gumboro virus (infectious bursal diseases/IBD) pathogenesis, since B cells of the bursa harbor the cytolitic phase of gumboro replication cycle. In the present study, inducible nitric oxide synthase (iNOS) associated with gumboro virus infection in the bursa of fabricius of broilers and provides extracts *Annona squamosa* (Sweetsop) as a leading immunostimulant were investigated.

This research using the 40 individuals one-day-old broiler chickens (DOC). Broiler chicken experiment was obtained from livestock breeding. The cage was used multiple systems Cages measuring 20 x 15 x 10 cm, each unit consisting of one head so that the number of cages all 40 units. Extraction of *Annona squamosa* (Sweetsop) is done at the laboratory of the Faculty of Pharmacy, Widya Mandala University. *Annona squamosa* (Sweetsop) used as test material was the result of reflux extraction methods using ethanol as solvent. The extract *Annona squamosa* (Sweetsop) with a dose of 10 mg/kg body weight, administered orally at the age of 7 days until the end of the experiment, namely the age of 35 days, given gumboro virus infection at the age of 20 days in a peroral dose of EID₅₀ 10⁷. The design was as follows: Group A: given the extract of *Annona squamosa* (Sweetsop) without infected gumboro virus; Group B: without a given extract *Annona squamosa* (Sweetsop) and without infected gumboro virus; Group C: given the *Annona squamosa* (Sweetsop) and infected with gumboro virus; Group D: without any extract *Annona squamosa* (Sweetsop) and given infected with gumboro virus. iNOS expression examination by immunohistochemistry method performed at the Laboratory of Faculty of Medicine, Airlangga University. The study began in June 2010 to October 2010.

From these results prove that the expression of iNOS was higher in the bursa of gumboro virus infected broilers without any extract *Annona squamosa* (Sweetsop) when compared to uninfected controls and provides *Annona squamosa* (Sweetsop) (p<0.05).

Keyword : iNOS, IBD, *Annona squamosa*, Bursa fabricius

INTRODUCTION

Gumboro virus is a non-enveloped, double-stranded (ds) RNA virus consisting of two segments, segment A (3.2 kb) and B (2.9 kb), encoding five proteins and belongs to the Birnaviridae family (Dobos *et al*, 1979; Kibenge *et al*, 1988; Mundt *et al*, 1997). Gumboro virus mainly affects young chickens from 3-6 weeks of age (Hoffmann and Lade, 1972). Although viral antigen has been detected in other organs within the first few hours of infection, the most extensive virus replication takes place primarily in the bursa of fabricius (Dobos *et al*, 1979). Activated dividing B lymphocytes that secrete IgM⁺ serve as target cells for the virus (Sharma *et al*, 2000; Hirai and Calnek, 1979). Viral infection results in lymphoid depletion of B cells and the destruction of bursal

tissues (Kaufer and Weiss, 1980), leading to an increased susceptibility to other infectious diseases and poor immune response to vaccines (Kibenge *et al.*, 1988).

Infectious bursal disease have not found the right treatment, because the gumboro virus attacks the lymphoid organs (thymus and bursa fabricius). Gumboro disease is preventable by vaccine, but the prevention of gumboro with any vaccine has not been efficient. It can be concluded that the vaccine can only handle 85-90% of gumboro cases (Hair *et al.*, 2000). The damage caused is usually permanent, because this lymphoid organ, initially had hypertrophic later developed into atrophy. Where is the result of atrophy of lymphoid organs are experiencing this performance of the organ to be not optimal.

The herb is one of the alternative precaution against gumboro. The difference between the vaccine and prevention through the use of herbal medicines, which are both situated on the effects. Medicinal plants with their natural properties will improve patient endurance especially in the immune system. Triadisti (2005) proved that infusa *Annona squamosa* has the ability as an antiviral against New Castle Disease Virus. *Annona squamosa* is also known to contain polyphenolic compounds, flavonoids, tannins, alkaloids and saponins, and is reported to have antiviral activity against EBV Early Antigen. Some chemical components in plants can be extracted *Annona squamosa* with ethanol, so ethanol extract of antiviral research needs to be done.

Avian macrophages play an important role as part of the innate immune system by producing cytokines and exerting phagocytic functions (Qureshi *et al.*, 2000). In natural gumboro infection, infected macrophages associated with the respiratory system are suggested to carry gumboro from the site of initial infection to the bursa of fabricius (Barrow *et al.*, 2003). Thus, it is possible that macrophages were involved in inhibition of gumboro replication. It is also possible that macrophages may have played a role in clearing gumboro-infected cells by phagocytosis (Djeraba *et al.*, 2000). Since in the present study the expression of iNOS was significantly higher in the bursa of gumboro infected chickens and correlated significantly with macrophage counts in the bursa, macrophages may have curtailed gumboro replication through NO production as has been described previously (Xing and Schat, 2000).

Despite this research the influence of ethanol extract of *Annona squamosa* leaves against the body's defense system overview broilers infected with gumboro virus (Infectious like HIV) particularly associated with the expression of iNOS has not been widely reported.

MATERIALS AND METHODS

This research using the 40 individuals one-day-old broiler chickens (DOC). Broiler chicken experiment was obtained from livestock breeding. The cage was used 40 systems Cages. Ethical Clearance for the present study was obtained by The Ethics Committee of the FKH Unair. Extraction of *Annona squamosa* (Sweetsop) is done at the Laboratory of the Faculty of Pharmacy, Widya Mandala University. *Annona squamosa* (Sweetsop) used as test material was the result of reflux extraction methods using ethanol as solvent. The extract *Annona squamosa* (Sweetsop) with a dose of 10 mg/kg body weight, administered orally at the age of 7 days until the end of the experiment, namely the age of 35 days, given gumboro virus infection at the age of 20 days in a peroral dose of EID₅₀ 10⁷. The design was as follows: Group A: given the extract of *Annona squamosa* (Sweetsop) without infected gumboro virus; Group B: without a given extract *Annona squamosa* (Sweetsop) and without infected gumboro virus; Group C: given the *Annona squamosa* (Sweetsop) and infected with gumboro

virus; Group D: without any extract *Annona squamosa* (Sweetsop) and given infected with gumboro virus. iNOS expression examination by immunohistochemistry methods performed at the Laboratory of Faculty of Medicine, Airlangga University. The study began in June 2010 to October 2010.

RESULTS AND DISCUSSION

From table 1 shows that the average expression of iNOS as the influence of *A. squamosa* in broiler chickens infected with gumboro (like HIV) differ significantly ($p < 0.05$), respectively, the group D ($27,00^a \pm 0,73$), group C ($15,00^b \pm 0,87$), group A ($7,00^c \pm 0,67$), group B ($5,00^c \pm 0,83$).

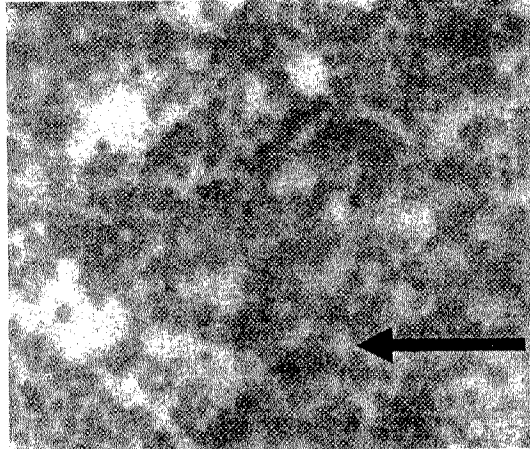


Figure 1. Immunohistochemistry analysis of the expression of iNOS of bursa fabricius of chickens infected gumboro virus (pembesaran 400X)



Figure 2. Immunohistochemistry analysis of the expression of iNOS of bursa fabricius of chickens without infected gumboro virus (pembesaran 400X)

Table 1. The average expression of iNOS as the influence of *A. squamosa* in broiler chickens infected with gumboro (like HIV)

Treatments	Average expression of iNOS
A : given the extract of <i>Annona squamosa</i> (Sweetsop) without infected gumboro virus	7,00 ^c ± 0,67
B : without a given extract <i>Annona squamosa</i> (Sweetsop) and without infected gumboro virus	5,00 ^c ± 0,83
C : given the <i>Annona squamosa</i> (Sweetsop) and infected with gumboro virus	15,00 ^b ± 0,87
D : without any extract <i>Annona squamosa</i> (Sweetsop) and given infected with gumboro virus	27,00 ^a ± 0,73

Different letters on the same column indicate significant differences (p <0.05)

Utilization of natural materials is one alternative to seeking new antivirals. It has been reported that the extract of *Annona squamosa* L. contains RIP (ribosome-Inactivating Protein) (Sulistyani *et al.*, 2009) because it can breakdown the DNA is supercoiled (Sismindari *et al.*, 1998). RIP shown to have antiviral effects as, in both plant and animal viruses (Barbieri *et al.*, 1993; Sulistyani *et al.*, 2009). Some chemical components in plants can be extracted *Annona squamosa* L. with ethanol, so ethanol extract of antiviral research needs to be done (Sulistyani *et al.*, 2009). RIP on some plants have been known to have antiviral activity with several possible mechanisms, including changing the permeability and facilitate entry of RIP into the infected cells, inactivation of ribosomes of infected cells to block protein synthesis and reduces viral replication (Barbieri *et al.*, 1993). Tannins in some plants also can inhibit the interaction of the host cell surface proteins and viral proteins, thus inhibiting viral attachment and penetration of virus into the plasma membrane (Moreira *et al.*, 2005 cited by Sulistyani *et al.*, 2009) or the tannins will bind well with viral proteins and host cell proteins to form complexes, thus preventing the virus adsorption process (Jasim and Naji, 2003 cited by Sulistyani *et al.*, 2009).

CONCLUSION

From this study proves that by giving *A. squamosa* will iNOS production decrease is due to the amount of virus replication has been declining in number due to the RIP (ribosome-Inactivating Protein) as an antiviral mechanism

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This is to certify that

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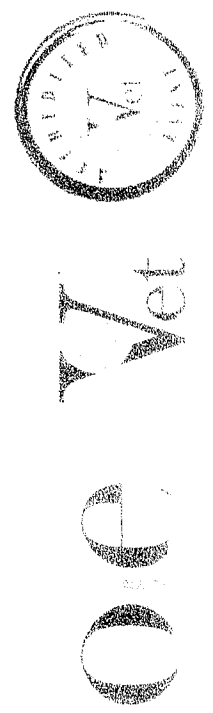
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EXPRESSION IN THE BURSA OF FABRICIUS OF
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