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Supplement 90



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Supplement 90

PROCEDING OF THE MINI WORKSHOP  
Southeast Asia Germany Alumni Network (SEAG)

*"Empowering of Society through the  
Animal Health and Production Activities  
with the appreciation to the Indigenous Knowledge"*

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## **Foreword**

The proceeding is a produced from papers collected during the Mini Workshop of Southeast Asia Germany Alumni Network (SEAG) on the topic of: “Utilization and Conservation of Natural Resources for Sustainable Development through the Harmonies of Human – Animal and Environment: Empowering of Society through the Animal Health and Production Activities with the appreciation of Indigenous Knowledge” held in Manado-Indonesia on May 3 - 5, 2007

Twenty one selected papers were presented in this proceeding from 35 participants which coming from 9 universities in Indonesia.

We would like to highly appreciate and deeply thanks to DAAD for their financial support as a main sponsorship in this Mini Workshop that it made the program very successfully conducted. The same thing is also going to SEAG-Indonesia and to the University of Sam Ratulangi Manado who fully supported this event as one of their scientific program.

Finally, we would like to thanks to all Steering and Organizing Committee who work very hard for the symposium including the preparation and finalization of this proceeding.

The Editor  
August, 2007

## Preface

Indigenous knowledge is the local knowledge that is unique to a culture or society. Indigenous knowledge is also known as local knowledge, folk knowledge, people's knowledge, traditional wisdom or traditional science. This knowledge is passed from generation to generation, usually by word of mouth and cultural rituals, and has been the basis for agriculture, livestock production, food preparation, health care, education, environment conservation and the wide range of other activities that sustain a society and its environment in many parts of the world for many centuries.

Recently research and theory development have focused on the resources and capabilities to satisfy customers and to manage in a highly competitive environment. Indigenous people use the resources available without depleting them. They use their intimate knowledge of plants, soils, animals, climate, and seasons, not to exploit nature but to co-exist alongside it. Plants provide food, medicines, pesticides, poisons, building materials; animals provide meat, clothes, string, implements, and oil.

Today, there is a grave risk that much indigenous knowledge is being lost and, along with it, valuable knowledge about ways of living sustainable both ecologically and socially. It needs to understand indigenous knowledge and its role in animal health and production systems from an integrated perspective that includes both spiritual and material aspects of a society as well as the complex relation between them. It is necessary to understand and to explore:

- The potential contribution that indigenous knowledge can make to empower the animal health and production
- The protection of indigenous knowledge and its utilization for the benefit of its owners and the communities particularly on its region
- Its place within the larger body of scientific and local knowledge systems.
- The cultural and moral values and systems of indigenous knowledge

This mini workshop is focused on the indigenous methods and management of animal health and production in relation of food supply, food security, processing and preservation, as well as the potential for

value added through other approaches and the use of modern (bio-) technology. This proposal is drawing up to explore the possibility to carry out mini workshop which will invite participants from SEAG (South East Asia Germany-alumni network) and part of important stakeholders on animal health and production. Increasing public awareness and encouraging in the contribution on *Empowering of Animal Health and Production through Valuing the Indigenous Knowledge and Practices* are the goal want to achieve after workshop.

The workshop focuses particularly on discussion in problem identification, policies, society and ecological impact of animal production activities, and the learning exchanges (best practices) of empowering animal health and production through valuing indigenous knowledge which has been accumulated over centuries has potential value for sustainable development. Therefore, specific objectives can be described as follows:

1. To empower SEAG-alumni become a target group who will appreciate and spread out indigenous perspectives on aspects between society – animal - environment relationships.
2. To appreciate, scientifically utilize and developing the role of indigenous knowledge and traditional ways of animal health and production in maintaining the sustainability and developing indigenous communities.
3. To identify opportunities for integrating relevant aspects of indigenous knowledge and approaches to the government policies that can support achieving multiple goals of animal health and production.
4. To disseminate the role of indigenous knowledge for better animal health and production purposes (transform knowledge into practice to reduce scientific and community gap).
5. To strengthen country collaboration between Germany and Indonesia through the role and contribution of alumni activities.

Organizing Committee  
August, 2007

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# **Maintaining Indigenous Knowledge for the Sustainability of Animal Welfare Beyond the Concept of National Park Model**

Adam Malik<sup>1)</sup>

## **Introduction**

Today, many **indigenous knowledge systems are at risk of becoming extinct** because of rapidly changing natural environments and fast pacing economic, political, and cultural changes on a global scale. Practices vanish, as they become inappropriate for new challenges or because they adapt too slowly. However, many practices disappear only because of the intrusion of foreign technologies or development concepts that promise short-term gains or solutions to problems without being capable of sustaining them. The tragedy of the impending disappearance of indigenous knowledge is most obvious to those who have developed it and make a living through it. But the implication for others can be detrimental as well, when skills, technologies, artifacts, problem solving strategies and expertise are lost.

### *What is indigenous knowledge?*

In many cases, the term "Indigenous" is defined as "local", "tribal", and "native". Also Indigenous People are often referred to distinctive tribes of minority groups, such as the Aboriginal Australians, American Indians, Sasak, Papua and so on. However, the term "Indigenous Knowledge" has a broader and more flexible scope of meaning.

Warren (1991) and Flavier (1995) present typical definitions by suggesting:

Indigenous knowledge (IK) is the local knowledge – knowledge that is unique to a given culture or society. IK contrasts with the international knowledge system generated by universities, research institutions and private firms. It is the basis for local-level decision making in agriculture, health care, food preparation, education, natural-resource management, and a host of other activities in rural communities (Warren, 1991).

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Indigenous Knowledge is (...) the information base for a society, which facilitates communication and decision-making. Indigenous information systems are dynamic, and are continually influenced by internal creativity and experimentation as well as by contact with external systems. (Flavier *et al.* 1995: 479)

Indigenous Knowledge systems are adaptive skills of local people, usually derived from many years of experience that have often been communicated through oral traditions and learned through family members and generations. The indigenous knowledge or local wisdom enables lifelong learning in society. It is not only strengthens the community's economic situation on the basis of self-sufficiency, but also moral values, and local culture among community people. However, if there is nothing done to promote the learning of local knowledge, our future generations will definitely not understand where we are in the world or even lose the root of their culture.

#### *Why is indigenous knowledge important?*

In the emerging global knowledge economy a country's ability to build and mobilize knowledge capital, is equally essential for sustainable development as the availability of physical and financial capital. (World Bank, 1997) The basic component of any country's knowledge system is its indigenous knowledge. It encompasses the skills, experiences and insights of people, applied to maintain or improve their livelihood.

Significant contributions to global knowledge have originated from indigenous people, for instance in medicine and veterinary medicine with their intimate understanding of their environments. Indigenous knowledge is developed and adapted continuously to gradually changing environments and passed down from generation to generation and closely interwoven with people's cultural values. Indigenous knowledge is also the social capital of the poor, their main asset to invest in the struggle for survival, to produce food, to provide for shelter or to achieve control of their own lives.

Indigenous knowledge about cultivated and wild species is rapidly being lost. Genetic information coded in wild strains and traditional or native crop varieties could be stamped out as intensive mono-cultural production favours the adoption of newer, high-yielding crops. Humanity's collective knowledge of biodiversity and its use and

management rests in cultural diversity; conversely, conserving biodiversity often helps strengthen cultural integrity and values.

To name but a few:

1. Traditional pastoralists as guardians of biological diversity. (i.e. managing the forest for National Park)
2. Some traditional cultures based on relationship with animals (i.e. taboos on selling female animals outside community, rules for passing on animals from one generation to next). This feeling of responsibility for welfare of animals significantly contributed to the gene pool.
3. Religious ceremonial use (i.e. devoting male animals to memory of an ancestor, white animals for cleansing bad omens, black animals for the curses).
4. Medicinal treatments by using partly of the animal (i.e. horn of Anoa for strengthen, horn of rhino for typical medicine, snake's skin for itching)

The underlying philosophy here is that nature's resources may be harvested as long as no species are threatened, and nature is left to operate with a reasonable balance of its own. The issue of non-cruel harvesting methods is identified as a valid concern, and is to be dealt with in the various cultural contexts in which it occurs. Documenting indigenous methods is an essential step toward conserving biodiversity. In this case, adapting animals to new and unfavourable environments requires care and determination.

Indigenous knowledge is not yet fully utilized in the development process. Conventional approaches imply that development processes always require technology transfers from locations that are perceived as more advanced. This has led often to overlooking the potential in local experiences and practices.

- The following experience from animal conservation program by Toro's local people may illustrate the consequences if local knowledge is not considered adequately. Result of research by Malik (2005) and Golar (2006) indicate that the society of Toro divide its region in 5 zone as according to its function that is: Zone of Wana Ngkiki exploited as place of animal habitat, Zone Wana as the obstetrical prop irrigate, Zone Pangale as traditionally hunting place, Zone of Pahawa Pongko to plant the annual crop and Zone of Oma Ntua to manage the crops. Moreover, there are also cultural values of conservation in society Toro posed at by custom order which prohibit the crop hewings exist in forest of Lore Lindu and custom initiative to do the cultivation in return the trees which is

cut away for special. Redemption of custom sanction in the form of buffalo, tray nor mbesa. It's concluded that the society of Toro own the local wisdom in exploiting and managing resources based on the existence experiences in around them, both in correlation with flora, fauna and also ecosystem as a whole.

- Commitment of Honey Bee Hunting in National Park of Lore Lindu.
- Conservation of Maleo based on community agreement of people from Wosu Village, Central Sulawesi that managing the harvest of Maleo's egg as well as the rules to sustain Maleo's population.
- In the area of Siberut Sumatera Utara, there is a habit of placing trust on the rule that all hunters have to distribute/devide its hunting for all countryside/entire people to avoid the hunter from a pain caused of curses. This belief lesson the hunt frequency so that give the opportunity of forest animal (pig and deer) to multiply.
- Tabla tribe who lives in the feet of Cyclops mountain had adopted the "Zoning" system in its nature resource utilization. Because of its geographical position, the nature resource utilization was arranged by pursuant to three Zoning corresponding to farm inclination. Those three systems arrange that:
  1. Area up to 300 meter above sea level utilized for housing, garden housing, traditional medicine gardening and all kinds of spices crop.
  2. Area on the height between 300 – 400 meter used for agriculture and crop rotation planting.
  3. Area's height between 400 – 500 meter designed for hunting and gathering of material substance for making boats. Above this level was trusted as a holy area dwelt by ancestors' souls. So, it can not be used.

#### *The role of the concept of National Park Model*

The first to answer is: What is a National Park Model? It can be concluded from the information which has been given by Directorate General PHKA as presented below:

- a diverse partnership of stakeholders and rights holders
- a large-scale working model of sustainable forest management
- a site for developing and applying new knowledge and technologies
- a focus for promoting ecologically sound forest management practices

- a consensus-driven partnership working with shared decision-making to achieve social, environmental, and economic sustainability in forest management
- a creator of on-the-ground solutions addressing local needs and global concerns
- a place where communities and traditional knowledge play a role in forest management
- a link in a network to facilitate an exchange of ideas and approaches to sustainable forest management

National Park has been assumed by the people as an economic resource for the people's welfare which has some demand consequences refer to its utilization. If the regional government come upon National Park as the resource of regional income in accordance with economic regional development, then, it makes the existence of National Park as the last chance to save the rest of the natural resource.

One of the important issues of **Dishut and Ditjen PHKA for 2005-2009** is managing the biodiversity resources by the activities of:

- Habitat / vegetation management
- Grassland management for animal feeding
- Animal population management
- Patterning facilities of drinking water and/or place for animal wallowing
- Cultivating and conserving trees as protection and feed source for animal.
- Controlling harming's animal population
- Enrichment/addition of indigenous plant or native animal
- Exterminate the disturbance vegetation and disorder animal

The conservation of biodiversity program through the concept of National Park Model concerning with animal welfare is focused on the

- Compilation/review regulation law about wild plant and animal
- Guidance and criteria of species management on the area.
- Campaign and extension
- Management of protected species
- Management and controlling the exploitation of unprotected species
- Increasing of product value and prima services
- Conflict handling between human and animal

In the year of 2009 those would be important aspect of strategically point by Ditjen PHKA with priority enforcement on:

1. Area stabilization
2. Identification and study of the public concern and eminently potency on the area which will be the trade mark of the National Park Model itself
3. Management area into participatory zoning
4. Compilation/review of planning management
5. Empowering natural resources utility regarding to cultivation, conservation, alternative energy and applied research activities.
6. Habitat rehabilitation, ecosystem restoration and managing dynamic population
7. Empowering of professional human resource
8. Developing of facilities management
9. Empowering on the investment opportunities from natural ecotourism employee as well as the environment utilization
10. Promoting and socialization of National Park conservation in according to obtain the appreciation and motivation of others
11. Attaining the opportunities of management collaboration of the national park.

## Conclusion

Indigenous knowledge is **relevant** on **three levels for the development process**.

- It is, obviously, most important for the **local community** in which the bearers of such knowledge live and produce.
- Development agents (CBOs, NGOs, governments, donors, local leaders, and private sector initiatives) need to **recognize** it, **value** it and **appreciate** it in their interaction with the local communities. Before incorporating it in their approaches, they need to understand it – and critically validate it against the usefulness for their intended objectives.
- Lastly, indigenous knowledge forms part of the **global knowledge**. In this context, it has a value and relevance in itself. Indigenous knowledge must be able to be preserved, transferred, or adopted and adapted elsewhere.

The development process interacts with indigenous knowledge. When designing or implementing a development programs or projects, three scenarios can be observed. The development strategy either:

- relies entirely or substantially on indigenous knowledge,
- overrides indigenous knowledge or,

· incorporates indigenous knowledge. Education in the globalization age should therefore be the balanced integration between global knowledge and indigenous knowledge. Therefore, modern science and technology must go hand in hand with indigenous or local knowledge for sustainable development in any community, international understanding, and peace and harmony of the world.

## References

Flavier, J.M. et al. (1995) "The regional program for the promotion of indigenous knowledge in Asia", pp. 479-487 in Warren, D.M., L.J. Slikkerveer and D. Brokensha (Eds) *The cultural dimension of development: Indigenous knowledge systems*. London: Intermediate Technology Publications.

Malik, A. 2005. Indigenous knowledge of Toro community in support of forest management in the area of National Park Lore Lindu, Subdistrict Kulawi, Donggala Regency, Province of Central Sulawesi. Mini Workshop of SEAG Indonesia Integrating Approaches to Achieve Multiple Goals in Sustainable Forest Management 19-20 September 2006, Hotel Horison, Semarang, Central Java.

Warren, D. M. 1991 "Using Indigenous Knowledge in Agricultural Development"; World Bank Discussion Paper No.127. Washington, D.C.: The World Bank.

World Bank, (1997) "Knowledge and Skills for the Information Age, The First Meeting of the Mediterranean Development Forum"; Mediterranean Development Forum, URL: <http://www.worldbank.org/html/fpd/technet/mdf/objectiv.htm>

# Indigenous Knowledge between Collapsion and Prospect of Genetic Conservation and Development

Ali M.A.Rachman<sup>1)</sup>

## Abstract

*This workshop is good start to review the performance Indigenous Knowledge (IK) in the past, its current issues and the possibility for tomorrow. Evidently, IK was a capable key to answer any problem in the community need. Yet, its one side is the chalenge of “the primitive” towardmodernization, another side its evolution is strong interrupted by global values. If so, it is a crucial in the community development today. So what can we do for the prospect of its sustainability? IK is a key strategy to understand human involvement in genetic resources of natural ecosystem of its evolution and revolution. Evidently, IK is used as a form in orally as accumulation of direct experiences. It had been transferred and distributed and collected as wisdom in the community by generation to generation in any variety of ethnicity of world wide people interaction with their specific natural ecosystem through exchange information flow. Today IK is not common in community practice and unluky mostly lag in modern publication. We belief the development policy by government is pairness based on a reason taken on rapidly people population growth and hunger. It is showing in economic activities lebel as single entity by calculation of benefit cost ratio perse. Such as activities, wisdom of IK experience as belong holism approach through generation estapet is exclude. According to this policy parity, IK is not effisien decision in New Era of human being. So, activities toward ecosystem run by revolution and its impact continually to deforestation genetic resources. Coordination between Government and legislation leaders have to wise to use IK to control community planed change so far. Such as valid and accuracy of historical data for animal herding and medicinal plant conservation for instance need IK. It is suggestion that conservation to discover way improvement from among the community themselves encouraged. In the meant time coevolution is suggestion pairness solving problems either evolution and revolution to reach welfare in order to use orientation of globalization market. Theoretically it is the way out to find sustainability of natural resources in ecosystem and deforestation protection. By coevolution activities of household economic parity toward genetic resources belong two sides. i.e IK practical involvement and new social trend values. The prospect of IK involvement in genetic conservation and development is higlight. If so, very much homework have to do. Let together with wise forests people and farmers hand by hand to indicate and to recover IK as components holism approach of the awareness by anthropologist and archaeologist.*

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Generally speaking that Indigenous Knowledge (IK) is coming from history of man interaction with his environment. Specific man and his specific environment had been produced a specific knowledge. It is used for wisdom references of the community in order to take decision making for welfare of the community as a whole.

Regarding to the above paragraph, IK is dynamic growth in various ethnicity and environment. In my experiences of fieldwork in human ecology it is begun in social economic context of technological change of sociological aspect in agriculture. IK in this term is blended between my Bogor Agricultural University experience of fieldwork among the Sundanese farmers of West Java as my first degree and later its combination with neo cultural evolutionary background of anthropological theory from American Scholar.

The IK has become reality in my mind since I was doing research among the aboriginal Malaysia for my Master degree in the year 1978-1980 then continue with East Coast tribal people of Sumatra for my Ph.D in 1980 - 1984. In the year 1986 I had chance to attend in "The World Archaeological Congress and I presented a paper of title "Energy and Sociocultural Evolution: Case on Kubu of Sumatra, Temuan Orang Asli of Human Ecology" in Semenanjung Malaysia and the Kubu Migrant from Sumatra to Semenanjung

Malaysia" (Cf. Ali M.A. Rachman in van der Leeuw & Robin Torrence (eds), Archaeological Congress Proceeding by Allen & Unwin; Southampton & London: 1986). Another finding of my fieldwork to support IK idea is information flow in Ecosystem through which I have written an article title "Traditional Information Capture and Environmental Knowledge" (Cf. Ali M.A. Rachman, Mimbar Sosek, Bogor: 1996) then introduce article "Mental Maps and Traditional Knowledge: A Field work Method in the year 1995 (Cf. Ali M.A. Rachman, in Jurq Schenider(ed), Indigenous Knowledge in Conservation of Crop Genetic Resources, CIP, Central Research Institute for Food Crops, Bogor CIP/CRIFC, printed in Indonesia).

IK in animal health and activities in this keynote as a point is not far from reflection history in anthropology and archaeology background of my experience basis known above. It is saying familiar as in term a patterns in its a direction evolution sequence as subsistence economic among small community either they are in village or in city. For foresters and non archaeologist –anthropologist or social economics it is hoped to utilize as awarenesses of idea in policy and politics.

Almost do not know accuracy data about development of civilization. Yet, suggestion by archaeologist it is clear about 40.000 years. Perhaps it is imagination date a progression from hunting to early agriculture to livestock husbandry. Only this is evident that occur between humans and animals, and to demonstrate that such relationships continue. The hunting of some animals and the keeping of others as valued companions was as much a part of human nature 10.000 years ago as it is today. Just as the domestic dog has the same behavioural pattern as the world, so the modern human probably differs little in his or her genetically inherited behaviour from the earlier *Homo sapiens*. It is only the development of culture and the ensuing pressures of social system that change.

Over the past 40 years understanding about human history and their interlocking with animal world made progress. Archaeology is no longer only the study of sherds and flints, and a whole new field of work, archaeozoology has become an essential part of the subject. This began with the simple identification of food remains on archaeological sites but it now encompasses almost all aspects of the physical life of humans and animals in the past, and reaches back to the evolution of the early hominids (Cf. Brock, J.C(ed) *The Walking Larder*).

In term limited data information about the past such as domestication, pastoralism and predation will be discussed in this keynote as long as it is support or a point in relationship the development today. Such as this point is urgent conflicting between evolution of IK and revolution as modern knowledge of biotechnology. Both IK and modern knowledge in ideally are not separation even data of it is missing link. They should have been connected and to combine as our world knowledge in term of coevolution.

Our note that mini workshop suggestion is good start to review small community of which IK be based on anthropology and archaeology explanation. In order to know what is IK in reality - the past and today community with its environment evolution should be understood. Perhaps some part of the IK useful for combination or selective values as a core Indonesian National Development verses the global values today.

### **Animal Health and Production of Subsistence Economic in relationship with Domestication, Predation and Pastoralism**

Animal had little to do with archaeology, except in artistic representation, and the relationships between humans and animals, if

thought about at all, were classed as anthropology or biology. To the non –biologist, wild animals were ferocious beast to be shot on sight, nature was red in tooth and claw, and natural history was pursued with a butterfly net.

Actually, archaeology is the history of human in their environment, and the history of their progressive travel higher and faster around it. Through at past the debris of human settlement and the present studies of human and animal behaviour, we can see that the master predator is indeed human behaviour as “an animal with unique consciousness and capability”, but an animal all the same.

These keynotes have tried making analogies the past and present of subsistence activities as of sources IK understanding possibility. In thisanalogies reconstructing past relationships between man and animals references can be made to the changes that occur in animals bone at different ages, in different condition. Example, modern and medieval data on tootheruprtion stages can be used to infer the ages of cattle and sheep mandibles in archaeological deposits (Halstead, P 1982). Regarding to thisexamples can be infer that archaeologist through possibility change of time, relatitively confident of their suggestion about the past. In another word artefact is one of reason method for modern knowledge to understand about the past.

Cultural anthropologist in this keynote is standing as sicientist who uses Archaeologist’s finding for knowled behaviour of the past for the present. In one hand it needs logical analogies in comparison. Besides anthropologist discovers the fact community behaviour life today.Through information flow of human ecology bakground - study of forest genetic resources sustainability and development is underlined.

## **1. Domestication**

Human being are the most highly social and gregarious of all the primates, and it may be postulated that their complicated patterns of social behaviour evolved from the necessity to provide food for a community or family group of early hominids that included helpless infants and aged relatives. The communal hunting of large ungulates for meat could have been part of the basis for the evolution of much human behaviour during the latter part of the Pleistocene. To progress from the hunting of wild animals to the herding of tame ones may seem a small step but it is, in fact, a very large one. Perhaps the role of IK is an urgent to know in human community as villager today because what separates hunting from herding is the concept of ownership of the animals among the settled human community.

The problem, what is continuing domestication's IK to biologist and anthropologist-archaeologist concern? IK record is never known formally, but orally inheritance among the community such as hunter gatherer may be to detect. Duck boy herding along sawah in Jalur Pantura of West Java is an another example of egg production still survive along the beginning of PELITA in the year 1969 (Five Period of Indonesia Development). The local species such as "hawarabatu rice genetic" in Cijengkol village of Sukabumi today disappear. Unlucky, IK by historical green revolution of Indonesia is not continuing in practice anymore. Is that possible to dig up IK by awareness of this mini workshop?

## **2. Pastoralism**

Pastoralism is subsistence pattern on the exploitation of herd animals and were all established during the prehistoric period, yet it still survive today. It is followed by nomadism together with hunting, and farming is at the next steps.

An assumption that IK could be a continuity from the hunting of wild animals to the following of herds, and hence to pastoralism. IK possibly is essays on the community transmitted. There is awareness that discussion on IK is broad-spectrum. Even today it is mostly replaced by modern knowledge i.e: knowledge in relationship with dependence on a few species of large mammals as resources diminished and the human population increased. There is change of climate, over-hunting, IK in its term of view is most collapse. As its substitution by modern knowledge - "biotechnology" to produce meat. Is there a reason so far IK as another side for answering the challenge for the future? A chance is possible just because the primitive community as known in anthropology as a wise community. Their knowledge understanding in their environment are evolve. Yet the problems very little Indonesian scientist interested to do research among the tribal people.

## **3. Predation**

It has been argued that all human activities involving the exploitation of animals are predation, and in social sense, parasitism. As soon as animals are herded or domesticated, human become protector except relationship between predator and prey. Some time this relationship is in form association each other getting benefits by widespread than it was in the wild.

Human cultural change relationship to animals is recorded by geologic time. In period of Palaeolithic were hunter-gatherer. In

Mesolithic they began to change to cultivation, storage of grain then keeping dogs with a number herd small animals. Full domestication began around 7000 year ago with never end hunting. Today, however even all modern man have been and are hunters, fishers and gatherers of wild food.

We believe that with animals predators, a balance is normally maintained by the control of environment and population dynamic so that prey species are not exterminated. Yet by human hunting at the end of Pleistocene, these controls appear to have broken down so that 'overkill'. Almost no detail information about the past history except little bit livestock in Java since 1850 (Cf. Barwegen, M). This is about genetic resource which are present as far as both large and small livestock are concerned, to the origine of existing breeds, and to their development after 1850. Since that date many types of cattle have been imported to Indonesia in the hope of 'upgrading' the local breed in various respects. One of its major aim was to produce stronger cattle for animal traction in the transfort sector. Infact, within 150 years the cattle gene pool within Java has twice been transformed to produce what appeared to be a new, more or less uniform type of cattle throughout the island.

The transformation cattle gene is as the following. First between Java cattle to Java-Ongole cattle. Second to an indeterminate new cross between the Java-Ongole and several introduced meat breeds. Its impact, valuable genetic resources originating from Java cattle have vanished. The accident is also occur to Madura and Bali cattle. This is an important awarness for Indonesian future, how to save genetic resources? Perhaps that is true by utilization governance coordination of power between government and legislation leader.

#### **4. Mental Maps in Subsistence Economic as Guard to IK**

Is genetics resources including animals is on balance in relationship to human activities in the future? The answer of this question is depend upon on several reason. The first look at human economic and population growth. In this point, susbsistence economic level is in common by simple techlogy. Perhaps we may say there is still in capability the regrowth of genetic resources. The other reason is regrowth is not possible any more if exploitation toward them by modern technology of which very efficient.

Generally, the major subsistence activities are conditioned by the most abundant food resources. In tundra, for example, where plant

food resources are scarce, emphasis is placed on hunting sea mammals and fishing in the coastal areas and caribou-hunting in the interior. In the tropical forest, at the end of spectrum, where the biomass of herbivores is low (Bourliere 1963), a great deal of emphasis is placed on gathering plant resources. The abundant of game in temperate and boreal areas seems to encourage intensive animal game hunting, as the evidence from prehistoric Europe suggests.

Accident such as killing by modern technology is continued. My experience in traditional groups The Kubu of Indonesia and The Temuan of Malaysia, mental maps role an important IK in association with their subsistence economic in the tropic. The community uses the maps for the special detection geography as environment where plant or animals familiar found for hunting and gathering.

The very interesting found during the fieldwork among the tribes is settlement modes. This settlement modes is a strategy management in economic to close with resources, to protect original settlement and shipment population from the original settlement to the other settlement in nomadic behaviour. In another word say this is subsistence economic among tribes and the small community.

Activities in every settlement modes showed that all stages of evolution that we have discussed. May be all of the modes performed as strategy in IK. Such as learning to domesticate monkey beside they killed for its meat and to tame bird as for collection beside they also grilled it. Appear in nomadic mode. Very little events, even never apparent activity to cure diseases of the any animal captured in every settlement mode by them. Only sometimes in a brief look they did care their animal as pet such as dog for their hunting. There is an important to note that technology in this moment is mostly simple but useful for killing such as blowpipes.

Let we know about events in each settlement modes as Rachman (in Jurg Scheneider,ed 1995:46-47) as it states below:

- (a) Settled Mode
- (b) Composite Mode
- (c) Dispersed Mode and
- (d) Nomadic Mode

In Settled Mode some of the population stay in their kampung or village. Activities are only performed within 5 kilometers of their village houses. Activities of this mode center on cultivation sites, such

as paddy fields, rubber plantation and orchards. Study of mental maps of this settlement pattern reveal that the community or the tribes familiar with large number of wild fruit species, some of which have been selected for domestication and cultivation in their gardens and orchards. This finding supports Rambo's view- as anthropologist and human ecologist (1979) that primitive man directly influenced the habitat modification of forest genetic resources through both domestication and dispersal. He suggests, as well, that this process of selection is part of primitive man's impact on genetic resources, that has occurred over time.

In Composite Mode some of the population stay in their village and it is a time of continuous shifting back and forth between a settled and nomadic pattern of living. It also has been seen that some population leave the village early in the morning for the forest or elsewhere, returning in the late afternoon. This activity is around 5-10 kilometers out of the village houses. In this mode indicate that although traditional communities have some cultivated land for their food production, they are still heavily engaged in activities related to the forest. These involve the collecting of various products, such as rattan, wild medicinal plants, and wild fruits, as well as the hunting of wild animals, such as pig, monkey, squirrel and goat.

In Dispersed Mode is indicated by durian season. At that time population dispersed to various areas for about 35 days in a year of their calendar. Small temporary hut is built in their orchard or in the forest, enabling the people to guard the ripening fruit and then collect it as it falls. A dynamic interaction with the external social environment has begun. For example, more intensive visiting by Chinese merchants, involving trade of forest products can be found elsewhere among traditional people, this may be an indication of an agricultural transition between closed and open ecosystem.

In nomadic living pattern, the populations carry out activities in the forest, mainly collecting and gathering wild products such as rattan (besides trading, it is also used for domestic purposes such as for making baskets, fish traps, animal traps, arms rings, decorative rings for their quivers and other homemade handicrafts). However, unlike the composite mode, this mode does not involve daily traveling back and forth between the forest and the village. Distances are further, so temporary shelters are built in the forest to save energy. When the supply of wild products in an area is exhausted, they move on to another area for collection. Mental maps are an example of which the traditional community or tribal people as indicated above known well about their

environment. At least two meaning knowled can be drawn from this field experience records. First IK is view as a strategic adaptation and second is view of food security policy by primitive. The author suggest the use mental maps is one practicing IK for a core contribution to modern knowledge.

## 5. Conclusion

IK is not clear in prehistoric time, except among the nomadic after agriculturalevolution begin around 10.000 years ago. Logically IK was transmitted from hunter – gathererof the tribes to small community of settled farmers. Such as subsistence modes of production of tribes is its traveling continuation from the past. Evidently, IK was excellent guard for the community welfare.

Such as song of “Nenek Moyang Ku Orang Pelaut is not known familiar any more today. It is about the sea fares of knowledge as an example IK that Indonesia’s ancestor as island people. We proud that our country is island connect each other as “Necklace around the Tropic”. Its wisdom link should be continued to the modern world. Unluky, even, it is ‘less spoilt’ by modern contact i.e. external social environment as global values.

Today, IK’s evolution is in between collapsion and prospect. Any one who cares about the future of Indonesia will have to want to think in the possibility IK as a core modern knowledge.

## 6. Implication

Regarding to the above narration and its conclusion images that is true to belief that Holy AlQuran to remind. For examples, Al Baqarah surat 2: “It is the Book; In it is guidance sure, without doubt, To those who fear Allah”. The fear of Allah, which, according to the writer of Proverbs in the Old Testament, is the beginning of Wisdom, restraint or guarding one’s tongue, hand, and heart from evil and hence righteousness, piety, good conduct. Allah who The Lord of The World to create all. “**Say Iqra’** - read under name of Allah”– meaning do research to discover the mystery of human being and two way back and forth relationship interaction toward environment will be reality as IK of the the tribes. So far the modern scientist wants to argue with subsistence of the tribes’sIK chalenge?

## References

Ali M.A.Rachman, 1996. Traditional information capture and environmental knowledge. *Mimbar Sosek. J of A & R S-E.9(2):36-52.*

Ali M.A Rachman, 1995. Mental Maps and traditional knowledge: A field work method in human ecology. *CIP&CRIFC. Bogor: p.45-48*

Ali M.A.Rachman, 1991. Social Integration and Energy Utilization: An analysis of the Kubu Suku Terasing of Indonesia and the Temuan Orang Asli of Malaysia. In, *Rambo,A.T &Katileen Gillogly(eds), Profiles in cultural evolution. Ann Arbor, Michigan:Anthropological papers, Museum of Anthropology , University of Michigan, N0.85:311-331.*

Ali M.A.Rachman, 1986. Energy and sociocultural evolution: case on Kubu of Sumatra, Temuan Orang asli of Semenanjung Malaysia and Kubu migrant from Sumatra to semenanjung Malaysia (In van der Leeuw & Robin Torrence(eds) *The social and economic contexts of technological change. Allen&Anwin, London: proceeding, TheWorld Archaeological Congress.*

Barwegen, M. 2003. Livestock in Java since 1850, in *Indonesian Environmental History Newsletter, No.18, Dec.2003*

Clutton-Brock, J(ed), 1989. *The walking larder. Pattern of domestication, pastoralism and predation. London: One World Archaeology No.2*

Halstead, P 1982. The animal bone. (A citation in Hodder 1982). *The present past. An introduction to anthropology for archaeologist.: p.111. London, T Batsford Ltd*

Rambo, A.T.1979. Primitive man's impact on genetic resources of the malaysian tropical rain forest. *Malays.Appl.Biol.8(1):59-6*

Rahman, Zainuddin A.1977. *The Temuan Ecosystem.* B.A Hons thesis, University Malaya. Manuscript.

Schneider, Jurg(ed).1995. *Indigenous Knowledge in Conservation of Crop Genetic Resources. Proceeding of an International Workshop. Held in Cisarua, Bogor, Indonesia January 30,-February 3, 1995.*

Syamsudin, A. 1989. *Konsep tata ruang lansekap kolonial Belanda: studi kasus di kota lama Jakarta.* Ir thesis, Bogor Agricultural University. Manuscript

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# Recent Progress in the Application of Reproductive Technology in the Indigenous Garut Sheep

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## Abstract

*Application of the reproductive technology in small ruminant such sheep is still limited as compared with that in cattle industry. Several hurdles exist to the application reproductive technology. These include such several unique biological features and anatomical structure. This restriction leads to the low of the application reproductive technology in small ruminant especially in sheep. On the other side, it is widely recognized that the application of the reproductive technology can accelerate in the increasing animal population and useful for animal conservation. Garut sheep is a potential indigenous sheep species that have a good performance and high reproductive ability. However, introduction of the reproductive technology in this species is still limited. It is therefore, the population of Garut sheep is still low. In this paper, recent progress in the application of the reproductive technology in Garut sheep to improve the reproductive capacity that has been performed in our laboratory such as estrous detection and synchronization, semen cryopreservation, artificial insemination, in vitro embryo production and embryo transfer are summarized. The feasibility of these technologies for practical application and the latest results of these new approaches are compared here. Factor affecting the current results and obstacles to be overcome in the near future will be discussed. Finally some relevance technologies to be applied are briefly summarized.*

*Key words: Garut sheep, reproductive, technology*

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## Introduction

Garut sheep is one of the indigenous Indonesian sheep that has high prolificacy and high adaptation capability in tropical environment (Aku *et al.* 2005). The high prolificacy was affected by a prolificacy gene (Inounu *et al.*, 1997). Moreover, Garut ram has higher body weight than other local rams. These good performances prefer by farmer not only for meat production but also for fighting championship in the traditional culture in West Java. According to the history, Garut sheep breed derived from the crossing between Indonesian local sheep, Merino from Australia and Kaapstadt from Africa, so is thus it is adaptable with the high temperature.

Because of these potency it is required an introduction of the reproductive technology to accelerate the increasing the population. However, the application of the reproductive technology is not develop well in this sheep species since many restrictions factors. This paper will summarize recent progress in the application of the reproductive technology which has been applied in this species for improving reproductive capability in order to accelerate the population.

### *First Generation Biotechnology: Artificial insemination and related technology*

#### **Estrus synchronization**

In group of animal, the female show randomly cycling. It is therefore the time of estrus cannot be predicted with certainty for an individual animal. This situation makes difficulty to apply some technologies such artificial insemination. In order to make efficiency the mating program, estrus synchronization is required. Estrus synchronization allows a group of females to predict the time of estrus which reasonably accuracy. Principally there are two basic methods of synchronization of the estrus cycle namely shortening the life span of the corpus luteum (CL) and extending the luteal phase (Hafez and Hafez, 2000). For the shortening the life span of the CL, generally used Prostaglandin F2 $\alpha$  to induce the premature regression of a cyclic CL (luteolysis). Meanwhile for extending of the luteal phase it is generally involved long term administration of a progesterone so that the CL regresses naturally during the period when progestogen being administrated. However, the administration of the PGF2 $\alpha$  in small ruminant such a sheep should be careful and should be known the reproductive status of the animal. Otherwise the abortion will be

happened in pregnant females. It is therefore the treatment with progestagen is more preferred than Prostaglandin F2 $\alpha$  since safety.

Several results have been conducted in Garut sheep to stimulate estrus synchronization by administration several hormones and laserpuncture. Rizal (2005) has applied Progesterone CIDR implanted for 13 days for estrus synchronization. Estrus was better stimulated by those treatments. Furthermore, Rizal (2005) reported that onset of estrus in Garut sheep was observed at average 33.6 h (range from 28-37h) after CIDR removal. Meanwhile Herdis (2006) compared estrus synchronization method using laserfuncture and the application of Progesterone-CIDR.

Result of those experiment revealed that estrus in Garut sheep can be induced by stimulation several acupuncture points by laser for 3 days. It was reported that onset of estrous was observed at 8.44 h in the luteal phase and at 16.22 h without known estrus phase after the end of laserpuncture application (Herdís, 2006). The onset of estrus was shorter after laserpuncture stimulation than CIDR-progesterone application. Furthermore, Herdis and Kusuma (2003) reported that onset of estrus was observed at 31,83h after 12 days CIDR application and 29,83 h after 12 days CIDR application combined with the application of estrogen on days 13. Meanwhile there was no significant difference in the number of estrus sheep after laserpuncture application. However, Setiadi 2007 (unpublished data) revealed that there were differences in the viscosity of the mucus of estrus.

### **Semen preservation and artificial insemination**

Artificial insemination as the first generation of reproductive biotechnology is generally use superior males. The technique was further improved with development of cryopreservation of semen and method in estrus synchronization. It is therefore, the availability of the sperm for the application of the reproductive technology is a crucial factor. Semen is also required not only for artificial insemination, but also for *in vitro* and *in vivo* embryo production. It is therefore the availability of the technology for semen cryopreservation play an important role in the application of the reproductive technology. The semen cryopreservation has been widely used to preserve gamete from several species. Our laboratory has been also developed several methods for Garut sheep semen cryopreservation. Rizal (2005) reported several research on the influence of extender and the component of the extender for improving semen cryopreservation by adding some component such glutathione. Yulnawati *et al.* (2006) tried to use natural

resources of tropical fruit for extender of Garut ram semen using of melon essence. Furthermore, Herdis *et al.* (2003) reported the beneficial effect of carrot essence for liquid Garut ram semen extender.

Garut ram semen can be collected not only from ejaculate but also from cauda epididymis. Rizal *et al.* (2005) reported that cauda epididymis sperm have good fertility after to be frozen. This result indicates that sperm genetic banking can be conducted even if the animal has died.

Effect of an additional glutathione on the fertility of ram semen has been conducted (Rizal 2005). Glutathione was given alone or in combination with B-carotene. He concluded that the additional glutathione 0.05% and 0.10% and β-carotene 0.002% in Tris egg yolk extender are an optimum doses to obtain a better quality garut ram frozen semen. Furthermore Rizal (2005) reported that the addition of glutathione and β-carotene can decrease melanoaldehyde level which is toxic for the sperm.

Based on the fertility, according to Rizal *et al.* (2005) who reported that the quality of frozen - thawed ejaculated sperm is better than frozen-thawed cauda epididymal sperm. However cauda epididymal sperm is still suitable for artificial insemination by cervical method. Table 1. showed the pregnancy rate and lambing of cervical insemination using frozen thawed ejaculate sperm and epididymal sperm (Rizal *et al.* 2005).

Table 1. Pregnancy and lambing rate of ejaculates and epididymal frozen-thawed sperm by cervical insemination (Rizal *et al.* 2005)

Frozen-thawed	Pregnancy Rate (%)	Lambing Rate (%)	Number of lamb		
			Single	Twin	Total
Ejaculates	7/12 (58.33) <sup>b</sup>	7/12 (58.33) <sup>b</sup>	5	2	9
Epididymis	4/9 (44.44) <sup>a</sup>	3/9 (33.33) <sup>a</sup>	3	-	3

Most authors reported that the most suitable extender of Garut ram semen was the extender based on Tris egg yolk. However, it was reported also that extender based on lecithin such as AndroMed® could be used also for Garut ram semen (Aku *et al.* 2005).

## *The second generation of the reproductive technology: Embryo transfer*

### **Embryo transfer technologies**

Embryo transfer has been widely applied in the dairy industry to improve fertility, productivity and to conserve the genes of high genetic value. However in small animal, the application of the embryo transfer is still limited. It is therefore, there are still many problems that should be handled such as the method for estrus synchronization and ovulation, method for embryo collection and transfer. Since it is difficult to manipulate of the reproductive organ, most of the embryo collection and transfer are use surgery method.

There is not much report in the application of embryo transfer technologies in Garut sheep. Setiadi *et al.* (2006) reported for the first time of embryo transfer in Garut sheep using fresh and frozen embryo derived from *in vitro* embryo production. Transfer of embryo was done by laparoscopic technique. They reported also for the first time lambing from this technique. However, since there is a few only sample, the pregnancy rate of this technique is still low only 16%. It is therefore the application of the embryo transfer in Garut sheep is still in the immature stage and required some improvement in related technologies.

However, the application of the conventional method of the embryo transfer with several steps such superovulation, and the flushing of the embryo from the uterus has not been conducted in Garut sheep. Although it has reported that flushing of embryo can be conducted without surgery in sheep (Holtz, 2005), but the application with surgery aid is still a choice.

## *Third generation of the reproductive technologies: *in vitro* embryo production*

### **Laparoscopic ovum picks up (LOPU)**

For *in vitro* embryo production, both male and female gamete should be available. For male gamete, several methods for sperm collection such as artificial vagina, electroejaculator, and massage have been developed. Since the cauda epididymis is known as sperm reservoir, the development of sperm collection from epididymis has been also developed. However, there is still some difficulties to obtain female gamete except in slaughterhouse or by surgery technique.

Recent development in ultrasound technology has been improved in follicle visualization and ovum pick technology especially in large animal. Nowadays it is not big problem to collect of oocytes for

*in vitro* embryo production. However, since unique anatomical structure of the reproductive organ and to small to be manipulated by hand through rectal palpation, the collection of gamete in this species is still problem. Development of laparoscopic technique in medicine to monitor internal organ has influenced the development in ovum pick technology in small animal. By a minimum surgery and with an aid of the laparoscopic equipment, follicles in the ovary can be easily visualized and easy to guide to be puncture with certain type of needle to obtain number of oocytes in live donor.

Setiadi *et al.* (2005) has been introduced lapasrocopic ovum pick up in Garut sheep for the first time. Although the number of oocyte recovery is still limited, it is interesting to note that ovum pick up in Garut sheep can be conducted weekly without big handicraft. Setiadi *et al.* (2005) also reported that gonadotropin should be administrated before LOPU to obtain huge number of oocytes. It is reported that minimum a ten visible follicle were obtained after gonadotrophin stimulation.

#### *In vitro* embryo production

Production of embryo *in vitro* is one alternative to conserve endanger species. The method also as choice when the conventional method of mating has a become problem. This technique makes unity of two gametes (sperm and oocyte) in the laboratory system under a certain medium. This process is usually included retrieval of eggs (oocytes) from the ovaries, *in vitro* maturation of oocytes, *in vitro* fertilization and *in vitro* culture of presumptive zygotes to the morulla or blastocyst stage of embryo development. Embryo produced *in vitro* may be either directly transferred into the uterus of recipient at day 7 or 8 estrous cycle or to be frozen for future transfer. However the efficient of *in vitro* embryo production is still low. Many factors are involved in the successful of the embryo production such sperm and oocytes qualities, culture media and skilled laboratory personnel.

The recent progress of *in vitro* embryo production in Garut sheep and several improvements on IVF techniques have been applied. Yulnawati *et al.* (2006) reported that oocytes can be collected from different status of ovaries. However, good quality oocytes were better collected from ovaries with corpus luteum and in the presence of dominant follicle. Furthermore these oocytes can reach higher maturation and fertilization rates than other status of the ovaries.

In the culture system to obtain huge number embryo, Setiadi *et al.* (2006) reported some improvement in using culture media for Garut sheep embryo production.

Tabel 2. Development of sheep embryo in different culture system

Media Culture	Number of oocytes	Cleavage rate (%)	Morulla rate (%)
TCM 199	125	(48/125) 38.40	(10/125) 8,00 <sup>a</sup>
TCM 199-	113	(51/113) 45.13	(29/113) 25.66 <sup>b</sup>
CR1aa			
CR1aa	97	(39/97) 40.21	(29/97) 29.90 <sup>b</sup>

<sup>ab</sup>, Different superscript letter in the same column indicated significantly difference (P<0.05)

Result of the experiment (Table 2) revealed that Medium CR1aa is better media for in vitro culture system of sheep oocytes in vitro. TCM can support only better in the early stage embryo development. The changing of culture media from TCM 199 to CR1aa can improve embryo yield.

#### *The fourth generation of the reproductive technologies: cloning and somatic nuclear transfer*

Until now the fourth generation of the reproductive biotechnologies has not yet been applied in Garut sheep species. The reason may be the application of the fourth generation of biotechnology is not yet crucial, since the population is still stand and not many farmers also need the technologies.

### **Conclusion**

Based on the collected data revealed that several modern reproductive technologies have been introduced to improve Garut sheep population such as estrus synchronization, artificial insemination, semen cryopreservation, *in vitro* embryo production even embryo transfer. However, the application is not routinely applied, since the owner Garut sheep only in small scale farmer and the technology is still expensive for farmer. Only certain farm has been applied the technologies to improve the population.

## References

Aku, A.S., T. Saili, M. Rizal, Herdis, B. Purwantara and M.R. Toelihere. 2005. Cryopreservation of Garut ram semen using lechitine-based extender. Proc. Intl Asia link Symphosium, Bali.

Rizal, M. 2005. Fertilitas spermatozoa ejakulat dan epididymis domba Garut hasil kriopreservasi menggunakan modifikasi pengencer Tris dengan berbagai krioprotektan dan antioksidan. Disertasi. Sekolah Pascasarjana IPB.

Hafez, E.S.E. and B. Hafez. 2000. Reproduction in farm animals. 7<sup>th</sup> Edition. Lippincott William and Wilkins. Philadelphia.

Herdis dan Kusuma. 2003. Penggunaan control internal drug release dan ovalumon dalam sinkronisasi domba Garut. Jurnal Sains dan Teknologi Indonesia.

Herdis, Yulnawati and M.A. Setiadi. 2003. The storage of Garut ram liquid semen with the carrot essence extender. Seminar Nasiotal X Persada. Pp 56 (Abstr).

Herdis. 2004. Optimalisasi pengolahan semen dan kajian pemanfaatan teknologi laserpuntur untuk sinkronisasi estrus induk betina dan peningkatanlibido pejantan domba garut betina (Ovis aries). Disertasi. Sekolah Pascasarjana IPB.

Holtz, W.H. 2005. Recent developments in assisted reproduction in goats. Small Ruminant Res. 60: 95-110

Inounu, I, B. Tiesnamurti, Subandrio dan H. Martojo. 1997. Laju ovulasi dan daya hidup embrio pada domba prolifik. Media Veteriner 4(3): 25-38

Rizal, M. 2005. Fertilitas spermatozoa ejakulat dan epididimis domba Garut hasil kriopreservasi menggunakan modifikasi pengencer tris dengan berbagai krioprotektan dan antioksidan. Disertasi. Sekolah Pascasarjana-IPB.

Rizal, M., M.R. Toelihere, T.L. Yusuf, B. Purwantara and P.Z. Situmorang. 2005. Fertility of frozen-thawed ejaculated and epididymal sperm of Garut ram. Proc. Intl. Asia Link Symphosium, Bali pp: 163-165

Setiadi, M.A., I. Supriatna dan A. Boediono. 2005. Follicle development after Gonadotrophin treatment in Garut sheep for laparoscopic ovum pick up. J. Agriculture and Rural Dev. Suppl. (JARTS) 83: 153 – 158

Setiadi, M.A., I. Supriatna dan A. Boediono. 2006. Bedah Minimalis melalui teknik laparoskopi untuk koleksi sel telur dari donor

hidup dan implementasi transfer embrio pada ternak domba.  
Laporan Penelitian Hibah Bersaing XII/3. LPPM-IPB, Bogor

Yulnawati, M.A. Setiadi dan A. Boediono. 2005. Maturation and fertilization rate of ovine oocytes collected from different status of ovaries. Proc. Intl. Asia Link Symposium, Bali pp: 199 – 222

Yulnawati, M.A. Setiadi dan A. Boediono. 2006. Penggunaan Medium CR1aa untuk produksi embrio domba in vitro. J. Ilmu Ternak dan Veteriner 11(2): 131-136

## **The Effect of Ambon Banana Stem Sap (*Musa paradisiaca forma typica*) on the Acceleration of Wound Healing Process in Mice (*Mus musculus albinus* ).**

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### **Abstract**

*The aim of this research is to find out the activity of banana stem sap (*Musa paradisiaca forma typica*) on the acceleration of wound healing process in the mice skin (*Mus musculus albinus*) based on gross and histopathological observations. Totally 45 heads of DDY mice ages 4-6 weeks were used in this study. The mice were divided into three groups, negative control (without treatment), positive control (*Bioplacenton*<sup>®</sup>) and banana stem sap. All mice were 1-1.5 cm incised on the dorsal back skin. Gross lesions were observed daily. On the 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days after the treatment, mice were euthanized and the skin samples were collected for further histopathological observation. The anatomical parameters were blood coagulation, dryness, attachment/narrowed of the wound and formation of the blood clot. The histopathological parameters were number of macrophages, neutrophils, lymphocytes, neo-vascularisation percentage of re-epithelialization and the thickness of fibroblast. All qualitative data were statistically analyzed using Analysis of Variance (ANOVA) and continued with Duncan Multiple Range Test. Gross lesion and the fibroblast thickness were observed and descriptively analyzed as a quantitative data. The result indicated that banana stem sap could promote the wound healing process. Gross lesion observation indicated that in the banana stem sap treatment the scab formation was faster than negative control and *Bioplacenton*<sup>®</sup>. On macrophages, neutrophils and lymphocytes observations, the statistical analyze showed that the banana stem sap treatment was significant increase ( $P<0.05$ ) than the negative control. The fibroblast thickness on the skin wound treated with banana stem sap was high and the formation was also faster than the negative control and *Bioplacenton*<sup>®</sup>. All result mentioned above indicated that Ambon banana stem sap was accelerated the wound healing process. Further study is required in order to clarify the mechanism of the sap on wound healing process as well as thir toxicity and possibility for use both in animal and human medicine.*

**Key words :** Banana, histopathology, mice, skin, stem sap, wound healing,

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## Introduction

Skin wound healing could be defined as a loss of integrity of the skin as a body's main barrier of outer surface. In the human and veterinary medicine, wound cases are very common such as due to surgery, traumatic, skin burn and others. Wound healing is influenced by many factors including the kind of medicine/ drugs uses. The use of drugs for wound treatment could be used in many ways and kinds; one of these kinds is the use of herbal medicine. It's already known that some plants could be used for wound treatment such as banana tree.

Skin is a main barrier for preventing the invasion of pathogenic microbes from the environment. Skin wound will facilitate the pathogenic microbes to enter the body and causing infection. The use of medicine/ drugs is aim to accelerate the wound healing process and to prevent from infection (Yahya 2005). Wound healing process could be divided in 3 phases, there are inflammation phase, proliferation phase (regeneration or fibroplasia) and re-absorption phase (maturation or tissue re-absorption). Parameters used to identify this 3 phase are inflammatory cells (macrophages, neutrophils and lymphocytes), neo-capilarization, re-epitilization and connective tissue (Sjamsuhidajat and De Jong, 1997; Kalangi, 2004).

Indonesia as a mega diversity country with 25.000-30.000 plant species has 6.000 species of medicinal plants (Kardono 2003). One of the potential plants to be explored as medicinal plants is banana plant. Banana tree is an indigenous plant of South East Asia including Indonesia (Munadjim 1983). This plant is growth well, easily and common found in a huge number in most South East Asian countries. People use this plant mainly for the fruit and leave; the stem is mainly used only for ruminant feed and some cultural activity, therefore the use of the stem is not yet optimally while the stem sap itself never been exploited at all. Satu dan Supriyadi (1995) stated that the banana stem contain serotonin, norepinephrine, dopamine, tannin, vitamin A, vitamin B and vitamin C that are very essentially for body in the wound healing process. Serotonin could increase the function of digestive tract, decreasing the process of inflammation and stimulate the skin cell regeneration. Priosoeryanto (2003) also explained that banana stem sap contain saponin, antrakuinon dan kuinon that functioned as antibiotic and accelerated the growth of cells on the regeneration process. This stem sap also increases the blood flow and stimulates connective tissue formation on the response of wound healing process. According to Djulkarnain (1998), Ambon banana stem sap could be used for pain

reliever and facilitate the increasing of absorbtion capability of medicine in the skin therefore could be use to treated contusio, skin burn, animal bit and as anti-inflammation.

Due to many beneficial activity of the stem sap that never been explore before, we conducted the present study in order to elabotae scientifically the activity of Ambon banana stem sap on the wound healing process.

## Materials and Methods

### **Banana Stem Sap Preparation**

The banana tree was identified as Pisang Ambon (*Musa paradisiaca* forma typica) for their species and variety in the Research Center for Biology, Indonesian Institute of Science (LIPI) Bogor. The stem sap was collected directly from the stem by cutting the stem with knife aseptically.

### **Laboratory Animal**

Totally of 45 head of mice DDY strain, 4-6 week old were used in this study. Mice were kept in the individual cage with the optimum environment and temperature (18-24<sup>0</sup> C). Mice were fed with a commercial feed and drinking water was given *ad libitum*. Adaptation period was done for 2 weeks.

### **Treatment of the Mice**

Mice were distributed into 3 groups i.e. negative control, positive control (Betadine<sup>®</sup>) and treatment (stem sap) groups. Each groups were then divided into 5 sub groups according to sampling day (day 3, 5, 7, 14 and 21). On the day 12nd, the hair on the back skin were shaved and 2 days later the incision about 1-1,5 cm were done. According to the groups, mice were treated topically with Betadine<sup>®</sup> or stem sap everyday. Daily observation of the gross lesion was done twice a day (morning and afternoon). On the desired day (3, 5, 7, 14 and 21) mice were euthanized using chloroform inhalation and the skin at the incision site and surrounding area were sampled for further histopathological process and observation..

### **Gross Lesion**

Gross lesion examination was performed daily from day 1 to day 21 by direct observing the wound for their blood coagulation,

dryness, attachment/narrowed of the wound and formation of the blood clot.

### **Histopathological Findings**

Histopathological observation was done by comparing the treated and un-treated groups on the parameters of the number of inflammatory cells, number of neo-capillary, percentage of re-epithelialization and the density of the connective tissue. Observation for the number of inflammatory cells were for macrophages, neutrophiles and lymphocytes. Inflammatory cells and neo-vascularisation examination were performed on 15 microscopical fields with 3 replicates using a light microscope. Percentage of the re-epithelialisation was done using a videomicrometer by calculating the ratio of the length area of the wound which covered by new epithelial cells with

Calculation of the re-epithelialisation according to DiPietro (2001):

$$\% \text{ Re-epitilisation: } \frac{\text{Length of the wound with new epithelial cells}}{\text{Total length of the wound}} \times 100 \%$$

Thickness of the connective tissue was examined by the intensity of the connective tissue with Masson Trichrome stained using a scoring methode as describe below.

**Table 1. Scoring Lesio for the Connective Tissue**

Grade	Parameters
+	The wound stil open with minimal density of connective tissue, the distance between connective tissue is loose.
++	The wound could stil open or partially closed with connective tissue density low to moderate in several areas.
+++	The wound could totally closed or minimally open with high density and compact of the connective tissue. Some loose area is still detected with formation of neo-capillaries
++++	The wound is totally closed with very high density and compact of the connective tissue

### **Data Analysis**

The data of inflammatory cells and formation of neo-capillary were statistically analyzed using ANOVA and followed by Duncan Multirange Test. Gross lesion and the densities of connective tissue were analyzed descriptively.

## Result and Discussion

### Gross Lesion

Wound healing process (daily observatio) of all groups was tabulated in Tabel 2 below.

Table 2. Gross Lesion of all Groups.

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
1	Wound still open, reddish, wet and blood clotting is present.	Wound still open, reddish, wet and blood clotting is present.	Wound still open, reddish, wet and blood clotting is present.
2	Wound still open, red to yellowish and wet	Wound still open, reddish, wet and white granule	Wound still open, reddish and wet
3	Wound still open, yellowish and minimal dry	Wound still open, minimal dry and reddish black granule	Wound still open and wet.
4	Wound still open and minimal dry	Wound still open and minimal dry	Wound still open and minimal dry
5	Wound still open, minimal dry and exudated	Wound still open but narrowed and minimal dry	Wound still open but narrowed and minimal dry
6	Wound still open, minimal dry and exudated	Wound narrowed with minimal scab formation	Wound still open and minimal dry
7	Wound still open and minimal dry	Wound narrowed with minimal scab formation	Wound still open and minimal dry
8	Wound narrowed, dry and with minimal scab fromation	Wound narrowed with minimal scab formation	Wound narrowed and minimal dry
9	Wound narrowed, dry and with minimal scab formation	Wound narrowed, dry and with moderate scab formation	Wound narrowed and minimal dry
10	Wound narrowed and dry with moderate scab formation	Wound narrowed and dry with complete scab formation	Wound narrowed, dry and with minimal scab formation
11	Wound narrowed and	Wound narrowed and	Wound narrowed,

	dry with complete scab formation	dry with complete scab formation	dry and with minimal scab formation
12	Wound narrowed and dry with complete scab formation	Wound started to closed and scab was detached	Wound narrowed and dry with complete scab formation
13	Wound started to closed and scab was detached	Wound was closed and scab trace still appeared	Wound started to closed and scab was detached
14	Wound was closed and scab trace still appeared	Wound was closed and scab trace disappeared	Wound was closed and scab trace still appeared
15	Wound was closed and scab trace still disappeared	Wound trace was disappeared	Wound was closed and scab trace still appeared
16	Wound trace was disappeared	Wound trace was disappeared	Wound was closed and scab trace was disappeared
17	Wound trace was disappeared	Wound trace was disappeared	Wound trace was disappeared
18	Wound trace was disappeared	Wound trace was disappeared and started hair growth	Wound trace was disappeared
19-21	Wound trace was disappeared and started hair growth	Wound trace was disappeared and started hair growth	Wound trace was disappeared and started hair growth

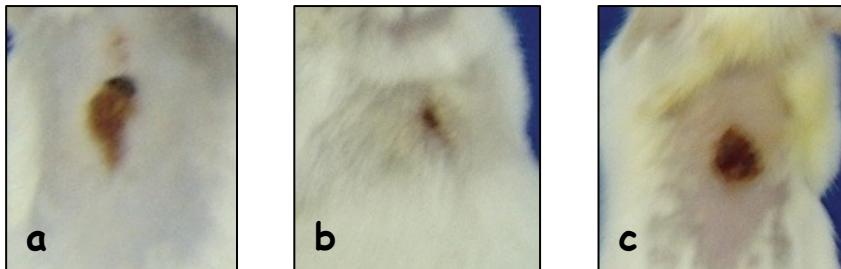


Figure 1. Gross lesion at day-7th post incision. a) Negative control; b) Banana stem sap and c). Betadine®

In the stem sap group, scab formation was started at day 7 post incision (PI), while in the Betadine® scab was formed at day 8 PI and in the negative control groups at day 10 PI. (Table 2). Scab is the manifestation of the granulation tissue, with earlier scab formation implicated that formation of granulation tissue was started earlier (Kalangi 2004). Detachment of scab and wound closed were appeared more early in the scab group, this condition implies that the skin was going back normally and the wound healing process entered the final stage. Disappering of the wound trace indicated that wound healing process was completed achieved.

Growth of the hair indicated that wounded skin were morphologically and functionally back to normal (Pinkus & Mehregan 1982). Growth of the hair was earlier detected on the stem sap group (at day 18 PI) this implies that treatment with stem sap caused wound healing process faster and better (Table 2).

### Neutrophiles

Neutrophiles is the one of blood component that play an important role in the earlier response to inflammatory, phagocytosis, killing the microbes and (Lever 1986).

The appearance of neutrophiles is acted as the first leucocytes response to the acute inflammatory in order to clean up the wound from contaminant microbes by phagocytic activity (Kalangi 2004). The number of neutrophiles on day 3rd PI in the stem sap group was significantly higher compared to the Betadine® and negative control groups ( $P<0.05$ ) as shown in Table 3. The high number of neutrophiles is indicated that the clean up and phagocytic activity was earlier occurred in the stem sap group compared to other groups.

Table 3. Number of Neutrophiles

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	233.33 ± 47.93 <sup>B</sup>	440.00 ± 46.94 <sup>A</sup>	203.33 ± 30.92 <sup>B</sup>
5	266.67 ± 118.09 <sup>A</sup>	256.67 ± 8.02 <sup>A</sup>	171.00 ± 40.26 <sup>A</sup>
7	146.00 ± 65.82 <sup>AB</sup>	122.67 ± 45.94 <sup>B</sup>	232.00 ± 32.92 <sup>A</sup>
14	88.67 ± 14.74 <sup>A</sup>	60.00 ± 11.27 <sup>A</sup>	66.33 ± 17.67 <sup>A</sup>
21	94.33 ± 16.072 <sup>A</sup>	59.00 ± 28.58 <sup>AB</sup>	37.67 ± 5.1 <sup>B</sup>

Note: The same alphabet (superscript) indicated no significant difference ( $P>0.05$ ).

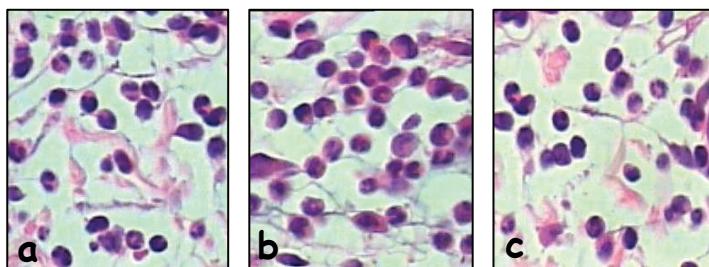


Figure 2. Number of neutrophiles day 3rd PI. a). Betadine®; b). Bananas stem sap and c).negative. Control. HE Staining. 1200X.

Kalangi (2004) stated that the present of neutrophiles in the wound is the first response of the body defense by fagocitic activity and will be decreased in line with cleanliness of the wound tissue. The high number of neutrophiles in the stem sap group at day 3rd and followed by gradually decreasing up to day 21st (Table 3) indicated that the wound is clean enough from contaminant microbes therfore the requirement of neutrophiles was also decreased. The presence of neutrophiles and macrophages on the wound area is sinergistic effect in order to clean up the wound (Nadesul 2003). The function of neutrophiles as phagocytic cells for clean up the wound tissue was very optimal in the earlier stage and then was gradually replaced by macrophages in the end stage of wound healing process.

## Macrophages

Macrophages is one of the bigger size of white blood cells with ability to digest the microbes, antigens and others substances which normally not circulated but present on the blood vessel associated - tissue (Yahya 2005). The function of macrophages is for fagositize and elimination (clean up) of tissue debris, killing of microbes and (Yahya 2005). The number of macrophages at day 5th PI on stem sap group was significantly high ( $P<0.05$ ) compared to other groups (Table 4). The condition mentioned above indicated that in the stem sap groups the fagocytic activity was high compared to the other two, and this implies the faster clean up of the wounded tissue by the sap.

Table 4. Number of Macrophages.

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	$212.33 \pm 54.05^A$	$262.00 \pm 64.13^A$	$231.33 \pm 52.52^A$
5	$240.33 \pm 35.22^B$	$485.00 \pm 63.02^A$	$274.00 \pm 15.10^B$
7	$411.67 \pm 60.93^A$	$285.00 \pm 8.66^A$	$385.33 \pm 91.49^A$
14	$82.67 \pm 23.07^B$	$139.67 \pm 30.66^A$	$132.67 \pm 12.90^A$
21	$76.33 \pm 32.02^A$	$80.67 \pm 43.98^A$	$38.67 \pm 9.29^A$

Note: The same alphabet (superscript) indicated no significant difference ( $P>0.05$ ).

Biologically, macrophages released the active substances such as vasoactive mediators, chemotactic, growth factors and enzymes including proteases (Kalangi 2004). In the wound healing process, macrophages formed a granulation tissue together with neo-capillary and connective tissue. The number of macrophages on the stem sap group was higher compared to Betadine® and negative control groups in every observation day (Table 4). The high number of macrophages will produce a lot of growth factors which will stimulate the growth of new cells (cell proliferation) and faster formation of granulation tissue that affected to the acceleration of wound healing process.

## Lymphocytes

In the immune systems, beside phagositoses, elimination of infectious or toxical agents is also by formation of antibody. The function of lymphocytes is as natural killer which could destroy alien substances or produce specific antibody (Guyton & Hall 1997).

Table 5. Number of Lymphocytes

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	13.67 ± 4.73 <sup>B</sup>	33.33 ± 9.87 <sup>A</sup>	14.00 ± 5.57 <sup>B</sup>
5	15.67 ± 1.53 <sup>B</sup>	27.67 ± 4.73 <sup>A</sup>	15.67 ± 1.15 <sup>B</sup>
7	9.33 ± 3.51 <sup>A</sup>	10.00 ± 6.08 <sup>A</sup>	10.33 ± 3.05 <sup>A</sup>
14	17.67 ± 9.50 <sup>A</sup>	14.00 ± 2.65 <sup>A</sup>	18.67 ± 14.50 <sup>A</sup>
21	24.00 ± 9.85 <sup>A</sup>	6.67 ± 4.160 <sup>B</sup>	14.67 ± 1.53 <sup>AB</sup>

Note: The same alphabet (superscript) indicated no significant difference ( $P>0.05$ ).

When specific lymphocytes were activated by antigen, lymphocytes will proliferate and produce antibody (Guyton & Hall 1997). The number of lymphocytes at day 3rd and 5th PI were significantly higher ( $P<0.05$ ) compared to the Betadine® and negative control groups (Table 5). The high presence of lymphocytes on the stem sap group indicated that lymphocytes act as body immune system together with neutrophiles and macrophages.

In the immune system mechanism, the presence of lymphocytes could be influenced by the presence of macrophages. Pathogenic microbes or substances will be phagocytized earlier by macrophages then their antigenic substances will be released into the cytosol. These antigens will be in contact with lymphocytes which stimulate the proliferation of lymphocytes (Guyton & Hall 1997). In this study, stem sap seems could stimulate the presence of macrophages which triggered lymphocytes to proliferate for producing antibody.

### Neo-capillaries Formation

Neo-capillary formation is one of a multistage mechanism in the wound healing process which a step of connective tissue remodelling (Vegad 1996). In this study, there was a non-significant difference ( $P>0.05$ ) on all groups in the neo-capillary formation.

Table 6. Number of Neo-capillary

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	39.67 ± 19.76 <sup>A</sup>	66.33 ± 26.03 <sup>A</sup>	65.67 ± 18.01 <sup>A</sup>
5	108.67 ± 30.14 <sup>A</sup>	163.67 ± 71.35 <sup>A</sup>	76.00 ± 14.53 <sup>A</sup>
7	155.00 ± 77.35 <sup>A</sup>	132.33 ± 58.20 <sup>A</sup>	213.67 ± 64.08 <sup>A</sup>
14	27.00 ± 14.53 <sup>A</sup>	44.33 ± 17.10 <sup>A</sup>	36.00 ± 1.73 <sup>A</sup>
21	40.33 ± 17.79 <sup>A</sup>	41.00 ± 29.51 <sup>A</sup>	20.67 ± 12.90 <sup>A</sup>

Note: The same alphabet (superscript) indicated no significant difference ( $P>0.05$ ).

According to Vegad (1996) neo-capillary was formed in the process of granulation tissue formation which started 24 hours PI and will be in the maximum at day 5th PI in order to fulfilled the nutrient intake for cells repair.

Increasing number of neo-capillary was early appeared on the stem sap group at day 5th PI (Table 6). This phenomenon indicated that stem sap stimulate the formation of neo-capillary therefore the nutrient intake is fulfilled sufficiently for the necessity of cell proliferation and healing process. At day 7th PI the decreasing requirement of nutrient in the affected tissue will also decreasing the number of vascular until the oedema process was disappeared (Vegad 1996). In the stem sap group, the decreasing number of neo-capillary was detected at day 5th PI while on the other groups was noticed at day 7th PI (Table 6), this condition indicated that decreasing of oedema reaction was quickly developed in the stem sap group.

### Re-epithelialization

Based on Stadelman *in* Kalangi (2004), re-epithelialization is one of the multistage mechanisms on wound healing process that include mobilization, migration, mitoses, and epithelial cell differentiation. These stages will re-conditioned the skin integrity. Mitoses and epithelial cell migration is functioned for re-conditioned of skin integrity. In our present study, there was no significantly difference ( $P>0.05$ ) on the re-epithelialization between groups (Tabel 7), this condition it seem due to no stimulation effect of stem sap on the process of re-epithelialization (Figure 3.).

Table 7. Percentage of Re-epithelization

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	0± 0 <sup>A</sup>	0± 0 <sup>A</sup>	0± 0 <sup>A</sup>
5	34 ± 15 <sup>A</sup>	45 ± 4 <sup>A</sup>	31 ± 20 <sup>A</sup>
7	56 ± 4.7 <sup>A</sup>	63 ± 32 <sup>A</sup>	64 ± 33 <sup>A</sup>
14	100 ± 0 <sup>A</sup>	100 ± 0 <sup>A</sup>	100 ± 0 <sup>A</sup>
21	100 ± 0 <sup>A</sup>	100 ± 0 <sup>A</sup>	100 ± 0 <sup>A</sup>

Note: The same alphabet (superscript) indicated no significant difference ( $P>0,05$ ).

Re - epithelization is a process of repairing the skin epithelial cells to facilitate the closing of wound on healing process. If re-epithelization develops quickly, the structure formation of epidermis layer will also quickly, therefore the repairing of the skin to become normal is also stimulated (Pinkus & Mehregan 1982). At day 5th PI, the percentage of re-epithelization in the stem sap group was higher compared to others, even statistically there was no significantly difference ( $P>0.05$ ), this implies that re-epithelization process was quickly developed in the stem sap group than the two others. At the day 14th and 21st there were a similar percentage on the re-epithelization process in all groups; this figure indicated that the body response to the repairing process of the wounded tissue has been maximum and optimally achieved.

### Connective Tissue

Connective tissue is a main component on wound healing process in order to increase and repair the skin/ tissue integrity (Kalangi 2004). In the stem sap group, the score of connective tissue density was high compared to the Betadine® and negative control groups (Table 8), this figure indicated that stem sap has an effect on stimulating the development of connective tissue which influences the strength of the repaired tissue (Figure 3).

Table 8. Connective Tissue Density

Day	Treatment								
	Betadine®			Stem Sap			Negative Control		
	+	++	+++	+	++	+++	+	++	+++
3	+	+	+	+	++	+	+	+	+
5	+	++	++	+	++	++	++	++	+
7	++	++	++	++	++	++	++	++	++
14	++ +	++ +	++ +	++ ++	++ +	++ +	+++	+++	+++
21	++ ++	++ +	++ +	++ ++	++ ++	++ ++	++++	++++	+++

Note : See table 1. for the scoring level

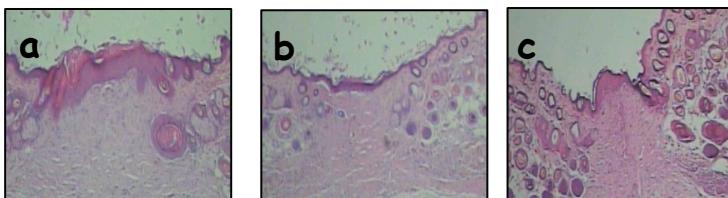


Figure 3. Microscopical appearance at day 14th PI. Negative control (a); Stem sap (b) and Betadine® (c). The number of inflammatory cells was decrease and re-epithelialization was completely occurred in the stem sap group. HE staining. Magnification 40X.

The main process of connective tissue growth will occurred at day 7th – 14th PI and then followed by gradually increasing of tissue repairing until the normal structure of the skin/ tissue was completely achieved (Kalangi 2004). At day 14th one of the replicant on the stem sap group has achieved the maximum level (++++) while the other groups achieved this level at day 21st PI (Table 8), this condition showed that the stem sap accelerated the skin/ tissue normalization.

## Conclusion

1. Banana stem sap accelerated the wound healing process.
2. Banana stem sap accelerated the detachment of wound scab
3. Banana stem sap increased the number of inflammatory cells

4. Banana stem sap did not increased formation of neo-capillary and re-epithelisation
5. Banana stem sap increased the growth of connective tissue
6. Banana stem sap is seem could be use as a medicinal substance for treated the wound healing
7. Further study for clarification of the mechanism of action as well as toxicological effect of banana stem sap on the wound healing process is required.

## References

Anonym. 2002. *Healing and Repair*  
<http://medweb.bham.ac.uk/http/depts/path/Teaching/foundat/repair/healing.html>. [15 Juni 2006]

.2005 *Pisang, Manfaat gizi Hingga Obat Luka*  
[http://www.republika.co.id/suplemen/cetak\\_detail.asp?mid=2&id=191805&kat\\_id=105&kat\\_id1=150&kat\\_id2=187](http://www.republika.co.id/suplemen/cetak_detail.asp?mid=2&id=191805&kat_id=105&kat_id1=150&kat_id2=187). [10 Juli 2006]

DiPietro LA *et al.* 2001. Wound Healing in MIP-1 $\alpha^{-/-}$  and MCP-1 $^{-/-}$  Mice. *American Journal of Pathology*. Burn and Shock Trauma Institute, Loyola University Medical Center. Maywood, Illinois;159:457-463.  
<http://ajp.amjpathol.org/cgi/content/full/159/2/457/F1>. [26 Mei 2006].

Fishman TD. 1995. *Phases of wound Healing*  
<http://www.medicaedu.com/phases.htm> . [10 Juli 2006]

Guyton AC, JE Hall. 1997. *Buku Ajar Fisiologi Kedokteran*. Edisi 9. Editor; Irawati Setiawan. Penerbit buku Kedokteran, Jakarta.

Kalangi SJR. 2004. *Peran Kolagen pada Persembuhan Luka*.  
[http://www.dexamedica.com/test/htdocs/dexamedica/article\\_files/kolagen.pdf](http://www.dexamedica.com/test/htdocs/dexamedica/article_files/kolagen.pdf). [15 Juni 2006]

Kardono LBS, N Artanti, ID Dewiyanti, T Basuki, K Padmawinata. 2003. *Selected Indonesian Medicinal Plants: Monographs and Descriptions*. PT Gramedia Widiasarana Indonesia. Jakarta.

Lever WF, SL Gundula. 1986. *Histopathology of The skin; Histology of the Skin*. Hlm. 8-36, Morphology of the Cells in the Dermal Infiltrate. Hlm. 47-55. J.B. Lippincott Company. Philadelphia.

Malole MBM, CSU Pramono. 1989. *Penggunaan Hewan Laboratorium*. Departement of Pendidikan dan Kebudayaan, Bogor.

Mayasari NLPI. 2003. *Kajian Patologi Khasiat Tanaman Lidah Buaya (Aloe vera) dalam Proses Persembuhan Luka Kulit pada Mencit (Mus musculus albinus)*. FKH IPB. Bogor.

Munadjim. 1983. *Teknologi Pengolahan Pisang*. Gramedia, Jakarta.

Nadesul H. 2003. *Biologi Sistem Kekebalan*. <http://www.medicastore.com/med/detail>. [15 Juni 2006]

Pinkus H, AH Mehregan. 1982. *A Guide to Dermatohistopathology 3<sup>rd</sup> Edition*; Normal Structure of Skin. Hlm 5-38, Ulcers. Hlm 193-200. Appleton Century Crofts, New York.

Priosoeryanto BP. 2003. *Penyembuh Luka di Sekitar Kita*. <http://www.kompas.com/kompas-cetak/0307/24/inspirasi/451512.htm>. [15 Juni 2006]

Satuhu S, A Supriyadi. 1999. *Pisang Budidaya, Pengolahan dan Prospek Pasar*. Cet. ke 10. Penebar Swadaya, Jakarta.

Soegiri J. 2006. *Tanaman Berkhasiat Indonesia*. Volume 1. IPB Press. Bogor.

Vegad JL. 1996. *A Textbook of Veterinary General Pathology; Healing and Repair*. Hlm. 134-153. Vikas Publishing House Put. Ltd, New Delhi.

Yahya H. 2005. *Kastil yang Terkepung: Tubuh Manusia*. [www.harunyahya.com/indo/buku/tubuh\\_003.htm](http://www.harunyahya.com/indo/buku/tubuh_003.htm) [ 15 Juni 2006 ]

# **Genetics Quality Improvement of Indigenous Beef Cattle Through Artificial Insemination Program in West Java**

Siti Darodjah Rasad<sup>1)</sup>

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## **Abstract**

Many concern from the government to increase productivity and population beef cattle in Indonesia, especially in West Java. One of the methods for breeding program is Artificial Insemination (AI). There were 10 indigenous breeds of beef cattle imported from abroad and was introduce with local cattle. That program was not followed by breeding program, it becomes negative consequence like inbreeding.

Artificial Insemination as a indigenous knowledge in Beef- and Dairy Cattle Farming, since 1953 have more advantage to improve productivity and population of Beef- and Dairy Cattle in Indonesia. A Case study in beef cattle farming in West Java in year 2003/2004 and 2006 with the goal to find out the performance reproduction of beef cattle through AI Program was performed. The data was collected from Bureau of Animal Husbandry of West Java Province. The aim of this research was to analyze performance reproduction of beef cattle in West Java Province supporting of AI program.

The result shown, that the performance reproduction of local beef cattle in West Java Province was good, with average Service per Conception (S/C) 1,66 (year 2003) and 1,67 (year 2006) and Conception Rate (CR) 61,37 %, (2003) and 71,59 % (2006). Average number of acceptor was increase in year 2003 (17.819) to 33.298 acceptors (2006), number of pregnancy with AI program was 9.084 cattles (2003); 18.626 cattles (2006) and average of parturition was 8.546 cattles (2003); 15.787 cattles (2006). The performance reproduction in two districts in West Java (Ciamis and Tasikmalaya) in year 2006 was better then others, S/C 1,85 and 1,45 with CR 71,97 % (Ciamis) and 60,23 % (Tasikmalaya). Beef cattle production through AI program in Ciamis and Tasikmalaya District was develop and increased the quality and income of the farmer, with the population of beef cattle in year 2006 as a acceptor AI was 5.625 cattles (Ciamis) and 4.010 cattles (Tasikmalaya). Production of beef cattle in Ciamis through AI program was increase (2003 – 2006) from 140 to 283 cattles per year. As a conclusion, the genetic potential of beef cattle in West Java province was increase and well developed through Artificial Insemination Program.

*Key words: AI, beef cattle, genetic improvement*

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## Introduction

Meat consumption program 2010 with the commitment “*Improving production strategic and farmer welfare in food consumption*”, it is not food consumption supporting to farmer welfare. This commitment means that production increase and farmer welfare is the role strategic of Government (Director General of Livestock), food consumption deficit are gradually still import.

Many factors should be concern to support succesfull of the program, especially improving and developing of current production. For instance, to increase of calving rate of beef cattle with the control of highly productized cattle female slaughtering, increasing Artificial insemination (AI) Acceptor, improving new setra development, protecting domestic market and banking policy.

In order to increasing AI acceptor, the government had target 3 million AI acceptors; with the assumption that existing program and current calving rate should be viability live 50% (1.5 million cows). It means with the proportional between male and female 50%, this program will be produce 750.000 male cattles per year.

Research by beef cattle in Sumatera, used sperm sexing straw, inseminated to resipient cow, shown by pregnancy diagnosis 2-3 month after insemination, 33 from total 116 cattle positive pregnant.

AI program in dairy cattle was done to increase the genetic value. Result of the research shown distribution of 360 female straw(X-straw) and 80 male straw (Y-straw), 64.5% resipient with X-straw and 53.8% resipient with Y-straw was positive pregnant.

Aim of this research was to evaluate performance reproduction beef cattle in West Java through artificial insemination program.

## Literature Review

Artificial Insemination (AI) is deposition of semen to female reproductive organ with AI tools. Ax et al. (2000) said AI is the most important single technique devised for the genetic improvement of animals, because a few select males produce enough sperm to inseminate thousand of female per year. The advantages of AI are enables the widespread use of outstanding sires with valuable genetics to any livestock operation, facilites progeny testing, leads to improved performance and potential of the national herd, permits crossbreeding,

enables use of frozen semen, and reduces risk of spreading sexually transmitted diseases

In Indonesia, AI program was introduced firstly by Prof. B. Seit in Bogor 1950. In 1953 AI program in Center Java for the Ongol Program and AI in Ungaran to increased milk production with FH Bull (Toelihere, 1985)

Introduction of Frozen semen in Indoensia was 1973 and used for beef and dairy cattle. Research about evaluation of AI program Java Cattle during 1972-1974, as a research collaboration between Directorate Jenderal of Livestock, Bogor Agricultural Institute and Animal Husbandry Faculty Padjadjaran University shown conception rate of frozen semen import was low (21.3-38.9%). Female infertility and disease caused of the lower of conception rate after AI technique. The government took over to improve AI organization, AI equipment and knowledge and capability of the farmer and AI instructor (Insemiantor)

## **Method**

Method of this research, as a case study in West Java, and the secondary Data was colleted from Yearly Report from Bureau of Animal Husbandry of West Java Province. The data about AI acceptor and performance Beef cattle through AI program in West Java Province. The data was analysed as descriptive analysis and the aim of this research was to evaluate the application and introduction of AI techniques to the reproductive performance of beef cattle in West Java and finally to improve the genetic quality of beef cattle.

## **Result and Discussion**

Evaluation of the collected data was shown in Table 1. Table 1 explained about recapitulation of AI acceptor and equipment supported to AI program in beef cattle in West Java Province during 2003 – 2006. The big potencial of AI program was conducted in south of West Java province. It is because of the population of beef cattle higher in south. In 2003 the population 223.818 cattles with the growth rate 8.73% was capable to reach the target. Supporting of AI program played important role. To see the performance of AI program to the performance reproductive during 2003 – 2006 it was shown in Table 2 and Tabel 3

about the performance of AI Program in 18 districts in West Java during 2006

**Table 1. Recapitulation of AI acceptor and equipment supported to AI Program in beef cattle in West Java Province during 2003 – 2006**

No	District	Location		Acceptors Population			AI Post
		SPIB	ULIB	2003	2005	2006	
1	Ciamis	3	14	2.435	4.057	5.625	3
2	Tasikmalaya	3	10	1.062	5.987	4.010	3
3	Garut	3	4	399	983	1.229	1
4	Cianjur	3	3	1.200	5.884	4.175	1
5	Sukabumi	3	8	800	1.745	2.741	1
6	Bogor	1	3		779	964	1
7	Karawang	3	3		144	514	1
8	Bekasi				608	842	
9	Purwakarta	1	2			1.616	1
10	Subang	2	6		1.552	2.692	2
11	Indramayu	3	3		741	363	1
12	Sumedang	6	21		3.651	5.016	6
13	Majalengka	1	3		1.102	2.745	1
14	Kuningan	1	5		282	260	1
15	Cirebon	2	9		400	245	2

*Bureau of Animal Husbandry of West Java (2003-2006)*

**Table 2. Performance of AI program of Beef cattle in West Java (2003-2006)**

No	Parameter	Year			
		2003	2004	2005	2006
1	No. of AI Acceptor (cattle)	17.819	21.368	25.400	33.298
2	No. of AI (dosis)	21.056	24.808	40.640	50.196
3	No. of Pos. Pregnant :				
	Cattle	9.084	10.942		18.626
	Dosis	15.056	20.336	20.320	31.169
4	S/C	1.66	1.86	1.48	1.67
5	CR (%)	61.37	53.81	55.43	71.59
6	No. of Calving (Cattle)	8.546	9.496	12.249	15.731

*Bureau of Animal Husbandry of West Java (2003-2006)*

After evaluation of the collected data it was shown that the performance reproductive beef cattle was increased during 2003-2006. It is possible because the AI program was an indigenous knowledge in West Java, especially in south of West Java. Generally, AI program have been increased the performance reproductive, which are the S/C and conception rate was increased from 2003 to 2006. The proportion calve

between male and female shown more male then female. This condition was good for feedlot, which are the growth rate male bigger then female.

**Table 3. Reproductive Performance of Beef cattle AI Acceptor in West Java (2006)**

No	District	AI Accep (cattle)	Σ IB (dose)	No. Pos. Pregnant		S/C	CR (%)		No. of Calving (cattle)		
				Cattl	dose		IB-1	Σ	J	B	Σ
I	Ciamis	5.625	7.087	3.297	9.126	1.85	71.97	78.31	1.58 3	1.33 9	2.922
2	Tasikmalaya	4.010	5.166	1.888	1.529	1.45	60.23	81.70	767	780	1.547
3	Garut	1.229	1.574	817	997	144	62.38	75.51	145	137	282
4	Cianjur	4.175	4.830	1.012	1.619	2.16	34.82	46.93	609	432	1.041
5	Sukabumi	2.741	3.418	1.208	2.993	2.38	48.11	67.98	219	180	399
6	Bogor	964	1.973	978	1.195	1.68	66.64	89.40	452	376	828
7	Bekasi	842	892	374	407	2.25	47.40	5109	144	171	315
8	Karawang	558	773	182	192	2.48	50.00	50.00	99	83	182
9	Purwakarta	1.616	3.112	980	1.101	1.33	79.84	89.01	568	557	1.125
10	Subang	2.692	4.721	1.553	3.098	1.55	19.92	50.13	632	620	1.252
11	Indramayu	363	469	221	433	1.74	46.05	72.70	124	177	301
12	Sumedang,	5.016	7.080	4.336	5.672	1.34	72.54	85.61	2.27 3	1.80 8	4.081
13	Majalengka	2.745	7.880	1.543	2.493	1.80	48.38	65.72	783	482	1.265
14	Kuningan	260	292	117	143	1.92	78.74	56.52	74	68	142
15	Cirebon	245	508	120	170	1.46	46.39	71.01	59	46	105
16	Bandung	-	-	-	-	-	-	-	-	-	-
17	Kota Tasikmalaya	72	167	59	12	2.75	65.28	81.94	30	39	69
18	Kota Banjar	-	-	-	-	-	-	-	-	-	-
	Total	33.29 8	50.19 6	18.626	31.16 9	1.67	56.30	71.59	8.53 1	7.25 6	15.78 7

*Bureau of Animal Husbandry of West Java (2006)*

The above data shown that there were two districts in West Java Province were better in the reproductive performance of beef cattle. There wereas Ciamis and Tasikmalaya district. In that area, beef cattle was developed since long time ago and the farmer have more experience with beef cattle. Supporting of AI program, becomes the performance reproductive beef cattle in that locations better and the beef cattle farming well developed. Table 4 shown the performance AI program in south of West Java during 2003-2006 below,

**Table 4. Performance AI program in south of West Java during 2003-2006**

No	District	IB Activity Result									
		Acceptor (cattle)		No. of Pregnant (Cattle)		S/C (times)		CR (%)		No. of Calving (cattle)	
		2003	2006	2003	2006	2003	2006	2003	2006	2003	2006
1	Ciamis	2.435	5.625	1.851	3.030	1,38	1,85	70,48	78,31	1.703	2.922
2	Tasikmalaya	1.062	4.010	221	1.392	1,33	1,45	58,36	81,70	156	1.547

*Bureau of Animal Husbandry of West Java (2003-2006)*

## Conclusion

The conclusion of the research and evaluation:

1. The improvement of genetic quality of beef cattle in West was done through AI program
2. Breeding system of beef cattle with AI could be increased the number of AI acceptor and to improve reproductive performance of beef cattle in West Java
3. Ciamis and Tasikmalaya dsitrect, were two district in West Java which have succesfull of AI program with the good AI eprformance, S/C, CR and calving rate

## References

Ax, R. L., M. R. Dally, B. A. Didion, R. W. Lenz, C. C. Love, D. D. Varner, B. Hafez, and M. E. Bellin. (2000). Semen Evaluation. In E. S. E. Hafez and B. Hafez. Reproduction in Farm Animals. 7<sup>th</sup>ed. Lippincott Williams & Wilkins. Philadelphia, Baltimore, New York, London, Buenos Aires, Hongkong, Sydney, Tokyo

Anonymous. 2003. Yearly Report 2003. Bureau of Animal Husbandry of West Java

Anonymous. 2004. Yearly Report 2004. Bureau of Animal Husbandry of West Java

Anonymous. 2005. Yearly Report 2005. Bureau of Animal Husbandry of West Java

Anonymous. 2006. Yearly Report 2006. Bureau of Animal Husbandry of West Java

Toelihere, M. R. 1985. Fisiologi Reproduksi pada Ternak. Gramedia (*in Indonesian language*)

## **The Utilization of Oil Palm Bud on Quail's Performance**

Daisy D. S. J. Tambajong<sup>1)</sup>

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### ***Abstract***

*Oil Palm bud is one of the wastes that be burned in oil palm plantation during replanting. Because of new policy from the government, i.e. zero burning in oil palm replanting area, in case to reduce waste in field we can use this waste for animal feedstuff. This study is concerned with the potential of oil palm bud utilization in feed of quail from 0 - 6 weeks of age. Completely Randomized Design with five treatments and four replications were used to analyze the data. These treatments are 0%, 4%, 8%, 12% and 16% Oil Palm bud in feed of Quails (T1, T2, T3, T4 and T5, respectively). The results showed that the highest average feed intake was found in treatment with 16% oil palm bud in feed, then 12%, 8 %, 4% and the lowest feed intake in treatment without oil palm bud (87.33, 86.62, 83.48, 83.15, and 82.19 g, respectively). The highest average daily gain is reach for T4 (24.24 g), and the lowest is for T5 (23.65 g). The highest feed conversion ratio found in T5 (3.64) and the lowest in T1 (3.32) However, according to the analysis of variance from these result showed no significant differences. From this result indicated that the utilization of oil palm bud in feed is as good as conventional feedstuff on feed consumption, average daily gain and feed conversion ratio from growing quails.*

**Key words:** *oil palm bud, feedstuff, quail*

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## Introduction

Oil palm (*Elaeis guinensis* Jacq.) is one of the most important estate crops in Indonesia. In 1968, the total area under oil palm cultivation was only about 120,000 hectares but in 1999 the area had extended to 2.8 million hectares, an increase of almost 24 times in 32 years and by 2006, no less than 5.6 million ha oil palm has been planted. In North Sumatra this plantation grow progressively, in early 1916 recorded with 1,272 ha, and in 2003 was reported that the total area of oil palm in North Sumatra was 3,712,878 ha (Statistic Centre Bureau, 2004). The ever-expanding oil palm cultivation in Indonesia and other tropical countries offers the possibility of an increased and constant availability of palm oil products for animal feed formulation. Since years, oil palm frond (OPF) has already given to animal in case to reduce the cost for feed. Its high fiber content makes it just given to ruminant (cattle, sheep and goat). Oil palm kernel cake (OPK), as by product that obtained after extraction of palm kernel of the fruits of oil palm tree is also use for the formulation of animal feed, and because of its high nutritive value, is considered to be excellent for ruminant and also suitable for swine and poultry (Zahari and Alimon, 2005; Jalaludin, 2007).

The oil palm will be replanting every 25 years, and if we calculating with the growth of this plantation during these years from 1916 to 2007 now we can say that thousand of hectares should be replant every year. Normally, these palms will be burned on replanting. After the new policy from the government, that no palm can be burned in field (known as zero burning), in replanting area besides frond, the terminal buds of this palm are also providing. These terminal buds are edible (Martin, 1999), however in North Sumatra this oil palm bud is not familiar as human food. On the other hand, unconventional animal feedstuffs which high nutritive value but not in concurrence with human food, as well as due to its availability and low cost, this oil palm bud from the replanting area is a good thing to fulfill in the needs of unconventional feedstuff.

Quail belong with chicken, pheasants and partridges to the family Phasianidae of order Galliformes of the class Aves of the animal Kingdom. Species or subspecies *Coturnix coturnix* are native to all continents except Americas. These birds were brought to domestication at about eleventh century to Japan from China across the Korean bridge as pets and singing birds. According to many written records between 1910 and 1941 the population of Japanese quail

(*Coturnix coturnix japonica*) increased rapidly in Japan (Howes, 1964, cit. by Shim, 2005). Adult male will be weigh about 100-140 g, while the females are slightly heavier, weighing from 120 – 160 g (Randall, 2005). Rural small holder farmers in North Sumatra under insufficiency conditions management raise quail, ether for its eggs or for its meat. On the other hand, quail need proper nutrition for growth, bone formation, feather development and health. Nutritionally adequate diets have a composite of ingredients that supply the bird with six essential nutrient classes. These nutrients include water, protein, carbohydrate, fats, minerals and vitamins. Each nutrient serves specific functions and must be provided by the diet in an adequate amount to promote tissue growth and maintenance. For a normal growth up to five weeks of age it needs 24-25% CP with 2900 kcal/kg EM, called as starter diet. Finisher diet (from 6 wk of age up to market) or layer diet (for egg production) should contain 18-19% CP.

Since there is no literature of oil palm bud as additional feed in animal diet available, the objective of this present study was to asses the effect of different level of oil palm bud in quail ration.

## **Material and Methods**

One hundred of Day Old Quails (DOQ) were randomly selected and assigned five per cage of 5 treatments with 4 replicates. Cage dimensions were 60 cm long x 40 cm wide x 20 deep. It consisted of 5 double-decker rows with 4 cages to a row in an open sided, naturally ventilated house. Proximate and other analysis is presented in Table 1.

The treatments consisted of a control group and four different levels of oil palm bud in the diet (0, 4, 8, 12 and 16% oil palm bud, respectively), contained mainly corn, soybean cake, fish meal, rice bran. All diets were calculated to contain similar levels of protein.

The oil palm bud from field was chopped with chopper machine, dried out, and then grinding. The analysis of oil palm bud meal (as fed-basis) is also present in Table 1. Through out the experiment, feed and water were provided *ad libitum*.

Analysis of variance test was conducted for each parameter using the completely randomized design. Performance data (feed consumption, average body weight gain and feed conversion ratio) were collected for the 6-weeks experimental period.

Table 1. Compositions and calculated analysis of experimental diets

<b>Ingredients</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>	<b>T5</b>
Palm oil bud <sup>1</sup>	0.00	4.00	8.00	12.00	16.00
Corn	46.25	43.97	41.35	37.78	33.74
Soybean cake	33.17	32.88	32.53	32.17	31.78
Fishmeal	15.00	15.00	15.00	15.00	15.00
Rice bran	3.08	1.60	0.52	0.10	0.07
Premix	0.75	0.75	0.75	0.75	0.75
Palm oil	1.75	1.80	1.85	2.20	2.66
<b>Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>
	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Crude Protein (CP)	24.11	24.11	24.11	24.11	24.11
ME (kcal/kg)	2,903. 67	2,905. 58	2,901. 17	2,901. 07	2,900. 27
Crude Fiber (CF)	4.95	4.86	4.81	5.11	5.55
Ca	0.94	0.96	0.97	0.99	1.01
P	0.75	0.77	0.78	0.81	0.84

<sup>1</sup> Analysis of oil palm bud: crude protein 12.65%, fat 3.66%, crude fiber 20.72%, Ca 0.45% and P 1.21% (analyzed by Animal Nutrition Laboratory, Animal Science Department, Faculty of Agriculture – North Sumatra University), ME 2630.10 kcal/kg (analyzed by Agency for Animal Husbandry Research and Development of Agriculture Department- Sei Putih)

## Result and Discussion

The average feed consumption, average bodyweight gain, and feed conversion ratio of tested quails during the early six weeks of age are presented in Table 2, Table 3 and Table 4, respectively. In average, the feed consumption of the tested quails are 84.55 gr/wk, or 12.08 gr/d. Quails received diet T5 has a higher consumption among the other four, and T1, which diet without oil palm bud in ingredients was at least in consumption (87.33 vs. 82.19 gr/wk). However, no significance different was observed among various treatment group during the experimental periods. Because of the similarity of nutritive value among the experimental diets, this higher feed consumption in diet T5 is believed as because of good perform and smell, as a flavor, that this oil palm bud has. According to Damron (2003) flavor might limit the decrease in feed consumption cause by ingredients such as blood meal, fish soluble and fermentation by products or dusty ground grains like wheat. Actually, feed intake can vary depends upon the metabolizable energy content of the diet, age, reproductive status and the ambient temperatures (Shim and Vohra, 1984). Furthermore, Shrivastavand and Panda (1982) reported that though raising the dietary energy from 2,600

to 2,800 kcal ME/kg did not influence the gain in weight, it affected significantly the efficiency of the utilization as the feed consumption was reduced significantly. Compared with the other treatments, the use of 12% oil palm bud in diet have an optimal body weight gain in comparing with the other experimental diets during this 6 weeks (Table 3). The use of 16% oil palm bud with 5.55 % CP in diet tends too much for raising quail, although the metabolizable energy is quite the same. The chicks eat more but showed less weight gain. According to Connell (1981), high-fiber diets are known to increase the rate of feed passage through the gastrointestinal tract and thus may result in a lowering of the actual ME values of the diets.

Table 2. The effect of different levels of oil palm bud in quail diets on average feed consumption, average body weight gain and feed conversion ratio

<i>Treatment</i>	<i>Replicate</i>				<i>Average</i>
	<i>R1</i>	<i>R2</i>	<i>R3</i>	<i>R4</i>	
T1	80.07	83.33	81.67	83.69	82.19
T2	81.07	82.69	86.86	81.98	83.15
T3	77.09	88.51	76.67	91.64	83.48
T4	80.70	84.32	87.82	93.66	86.02
T5	82.23	87.96	90.26	88.87	87.33
<i>Average</i>					84.55

Table 3. The effect of different levels of oil palm bud in quail diets on average body weight gain

<i>Treatment</i>	<i>Replicate</i>				<i>Average</i>
	<i>R1</i>	<i>R2</i>	<i>R3</i>	<i>R4</i>	
T1	22.22	23.31	26.15	23.54	23.80
T2	23.97	23.23	24.17	24.16	23.88
T3	22.67	25.38	22.41	26.01	24.12
T4	22.29	25.18	23.57	25.93	24.24
T5	22.84	23.67	24.29	23.79	23.65
<i>Average</i>					23.94

Table 4. The effect of different levels of oil palm bud in quail diets on feed conversion ratio

<i>Treatment</i>	<i>Replicate</i>				<i>Average</i>
	<i>R1</i>	<i>R2</i>	<i>R3</i>	<i>R4</i>	
T1	3.54	3.31	3.04	3.37	3.32
T2	3.29	3.56	3.42	3.37	3.41
T3	3.57	3.25	3.57	3.44	3.46
T4	3.60	3.47	3.56	3.36	3.49
T5	3.74	3.63	3.50	3.70	3.64
<i>Average</i>					3.46

Feed conversion, expressed as grams of body weight gain per gram of feed that chick eaten, was not significantly affected by inclusion of oil palm bud in the diets, although the higher the level of oil palm bud the bigger the feed conversion rate. Yeong *et al.* (1981) reported broilers fed with palm kernel meal (PKM) up to 40% in isonitrogenous and isocaloric diet gave no significant difference in feed intake and daily weight gain of any diets, however, the feed conversion ratio significantly improved when diets containing lower level of palm kernel cake. Perez (2000) reported although PKM is a high-fiber, low energy feedstuff but it can be used up to 40% with little effect on performance of hens. It might be, if we raised the level of oil palm bud in diets would have an important effect on raising quail performance.

Since feed constitutes 60-70% investment at the farm, for deriving maximum benefit out of quail farming it is necessary to feed a balanced ration which has all the nutrients in necessary proportion. Many additional ingredients can be used, but ingredient substitutions require reformulation to adjust for nutritional variations in feedstuffs. From a utilitarian point of view this study, the concept of feeding oil palm bud from replanting area of oil palm plantation to quail is feasible. Oil palm bud can be used up to 16% as an alternative for unconventional feedstuff in raising quail diet, so far the crude protein and metabolize energy in diets is adequate.

## References

Biro Pusat Statistik. 2004. Statistik Kelapa Sawit 1998 – 2003.

Connell, A.M. 1981. Dietary Fiber. In Physiology of Gastrointestinal Tract. L.R. Johnson, Ed. Raven Press, Ny. Pp. 1291-1299

Damron, B.L. 2003. Feed Flavoring For Poultry. Publication of A Series Of The Animal Sci.Dept. Florida Extension Service, Inst. of Food and Agricultural Scie. Univ. of Florida.

Jalaludin. 2005. Integrated Animal Production In The Oil Palm Plantation. In Livestock Feed Resources within Integrated Farming System. Malaysia.

Martin, F. 1999. Multipurpose Palm You Can Grow. The World's best. Ed. Craig. Eleventh.

Perez, J.F. A.G. Gernat and J.G. Murillo. 2000. The Effect of Different Levels of Oil Palm Kernel Meal in Layer Diets. Poultry Science 79:77-79.

Randall, M. 2005. Raising Japanese Quail. Agfact.A5.0.6. Nsw Deparment of Primary Industries.

Shim, K. F. 2005. *Coturnix Quail. The Nutrition and Management of Japanese (Coturnix) Quail in the Tropics.* That Quail Place.

Shim, K. F. and P.Vohra. 1984. A Review of the Nutrition of Japanese Quails. *World's Poultry Sci. Journal*, 40:261-274

Shrivastavand, A.K. and B. Panda. 1982. Effect of Increasing Calorie- Protein Ratio during Growing Period (4-5 Week) Chemical Composition of Quail Meat. *India J. Poult. Sci.*, 17:253-256

Yeong, S.W., T.K. Mukherjee and R.I. Hutagalung. 1981. The Nutritive Value of Palm Kernel Cake as Feedstuff for Poultry. *Proc. of Nat. Workshop of Oil Palm by Product Utilization*, Kualalumpur Malaysia.

Zahari M.W. And A.R. Alimon. 2004. Use of Palm Kernel Cake and Oil Palm by -Product in Compund Feed. *Palm Oil Developments No.40. Mpob- Malaysia*

# Improving Cocoa Pod Quality by Urea, NaOH and Cocoa Pod Ash Alkali Treatments for Ruminant Feedstuffs

Despal<sup>1</sup>

## Abstract

Several alkali sources have been tested as agents to improve cocoa pod (CPs) quality. They are UREA ( $U1 = 10$ ,  $U2 = 20$  and  $U3 = 30$  g/kg fresh substance of CPs), NaOH ( $N1 = 2$ ,  $N2 = 4$  and  $N3 = 6$  g/kg fresh substance of CPs) and cocoa pod ash solution/CPA ( $A1 = 5$ ,  $A2 = 10$  and  $A3 = 15$  g/kg fresh substance of CPs). As controls, cocoa pod fresh (F) and cocoa pod incubated without alkali (Silage = S) have also been tested. Proximate compositions, cell wall constituents and in vitro gas production of untreated and treated CPs have been determined. The urea treatments are superior compare with other treatments in improving chemical compositions of CPs by reducing ash and crude fibre (CF) contents and increasing the crude protein (CP) content. Silage treatment reduce the ash and CF contents too, however, there is no improvement of CP. The same trends are also shown by the fibre analysis of cell wall constituents. The gas production after 24 h incubation of treated CPs showed that urea treatments ( $U1$  and  $U2$ ) and silage gave the best results. Estimate OMD and ME of  $U2$  and  $U3$  improve by 30% and ME by 49% compare to S and F. In vitro gas production ( $Gb$ ) as a respond to urea application ( $U$ ) followed equation of  $-0.0016U^3 + 0.0489U^2 - 0.2757U + 11.51$ . The  $Gb$  peaked at urea level about 17 g/kg fresh substance CPs (w/w).

**Keywords:** cocoa pod, alkali treatments, urea, cocoa pods ash, NaOH, in vitro gas production

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## Introduction

Availability of high quality forage is a major problem in ruminant production in tropical countries particularly during the dry season. At this season forage is expensive and out of reach to many farmers. As a result, there is over-slaughtering and selling of ruminant livestock when the production cost is elevated by the price of forage. Utilization of other abundant agricultural by-products is one of the alternatives to substitute expensive forage to keep the ruminant body weight losses at the minimum level.

Cocoa pod, a by-product of cocoa plantation has a great potential for ruminant feedstuff to substitute forage in dry season. However, as a late mature crop residue, the pod has low quality feed and need pre-treatments to improve its utilization by ruminant.

The use of physical, chemical and biological treatments to improve the quality of agricultural by-products has been reviewed by FLACHOWSKY et al. (1999). Previous study in our laboratory concluded that simple chemical treatment is as effective as biological treatment to improve cocoa pod quality (Laconi, 2000).

Alkali agents such as NaOH, urea and cocoa pod ash (high in potassium content) can cleave lignocelluloses into lignin and cellulose or hemicelluloses. They have been reported to be effective in improving fibrous feed quality (SCHIERE & IBRAHIM, 1989; SUNDSTØL et al, 1993; CHENOST & KAYOULI, 1997). In addition to improving digestibility, urea treatment may also supply N for microbial growths.

Application of alkali agent is species specific. So far, there is limited information on alkali agent application level on cocoa pod quality improvement. This study is focused to find an alkali treatment and their level on cocoa pod which give the best quality improvement for ruminant feedstuff.

## Methods

The cocoa pod from PTP VIII Rajamandala plantation has been used in this study. The treatments are UREA (U1 = 10, U2 = 20 and U3 = 30 g/kg fresh substance of CPs), NaOH (N1 = 2, N2 = 4 and N3 = 6 g/kg fresh substance of CPs) and cocoa pod ash solution/CPA (A1 = 5, A2 = 10 and A3 = 15 g/kg fresh substance of CPs). As controls, cocoa pod fresh (F) and cocoa pod incubated without alkali (Silage = S) have also been tested. The pods are sliced to 0.5 cm wide and added with alkali agents (except treatment F and S), homogenized and incubated an-aerobically for 2 weeks (except treatment F). The pods are then dried

and milled pass to 0.5 mm screen. The pod meals are tested of their proximate (dry matter, ash, crude protein, crude lipid, crude fibre) compositions based on Neumann and Bassler (1997) methods. Fibre contents followed Van Soest and Robertson (1979) procedure to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Gas production (Gb) of 24 h in vitro incubation (Menke et al., 1979) together with proximate composition have been used to estimate OMD (%) according to equation:  $14.88 + 0.889 \text{ Gb (ml)} + 0.045 \text{ CP (g/kg DM)} + 0.065 \text{ ASH (g/kg DM)}$  and to estimate ME according to equation:  $1.242 + 0.146 \text{ Gb (ml)} + 0.007 \text{ CP (g/kg DM)} + 0.224 \text{ XL (g/kg DM)}$ . All treatments are completely randomized and replicated three times. Mean value of treatments are tested according to Varian analysis and followed by Tukey's test.

## Results

The proximate analyses of treated cocoa pod are showed in table 1. The urea treatments generally improve CPs chemical composition better than others alkali sources. Urea treatments reduce about 25% of ash and 8% CF. Silage (treatment with incubation without alkali source) reduced about 21% ash and 20% of CF. However, on the silage treatment, there is no increasing of CP. The increasing of CP followed equation of  $7.823 + 1.1083 \text{ Urea (g/kg CPs)}$ .

Table 1: Proximate analysis of treated cocoa pod

Treatments	Dose	DM	ASH	CP	XL	CF
	g/kg	%	%	%	%	%
Fresh		87.65	7.88 <sup>b</sup>	9.34 <sup>b</sup>	0.54 <sup>b</sup>	52.30 <sup>c</sup>
Silage	0	89.44	6.19 <sup>a</sup>	8.68 <sup>ab</sup>	0.48 <sup>ab</sup>	42.09 <sup>a</sup>
CPA	5	87.41	8.34 <sup>b</sup>	7.85 <sup>ab</sup>	0.50 <sup>ab</sup>	49.59 <sup>bc</sup>
	10	87.09	9.32 <sup>bc</sup>	7.89 <sup>ab</sup>	0.63 <sup>b</sup>	50.83 <sup>bc</sup>
	15	86.67	10.39 <sup>c</sup>	7.96 <sup>ab</sup>	0.47 <sup>ab</sup>	49.32 <sup>bc</sup>
NaOH	2	88.88	7.89 <sup>b</sup>	7.49 <sup>a</sup>	0.43 <sup>ab</sup>	46.30 <sup>ab</sup>
	4	87.32	9.59 <sup>c</sup>	7.33 <sup>a</sup>	0.34 <sup>ab</sup>	46.27 <sup>ab</sup>
	6	87.16	10.64 <sup>c</sup>	7.33 <sup>a</sup>	0.27 <sup>a</sup>	47.67 <sup>bc</sup>
Urea	10	88.88	6.32 <sup>ab</sup>	19.36 <sup>c</sup>	0.40 <sup>ab</sup>	47.18 <sup>bc</sup>
	20	88.06	5.81 <sup>a</sup>	26.51 <sup>d</sup>	0.39 <sup>ab</sup>	49.98 <sup>bc</sup>
	30	87.96	5.49 <sup>a</sup>	43.24 <sup>e</sup>	0.48 <sup>ab</sup>	46.83 <sup>b</sup>

CPA = Cocoa pod ash. Different superscript at the same column shows the statistically different at  $p < 0.05$

Urea treatments reduce ADF content more than 10%; however not significantly increase the NDF content. In contrast, the silage treatment did not success in reducing ADF content but improve 9% of NDF content. There are no significant improvements of cell wall constituent by other treatments.

**Table 2: Van Soest analysis of treated cocoa pod**

Treatments	Dose g/kg	ADF	NDF
		%	%
Fresh		69.52 <sup>b</sup>	79.48 <sup>ab</sup>
Silage	0	62.15 <sup>ab</sup>	82.04 <sup>ab</sup>
CPA	5	69.23 <sup>b</sup>	86.81 <sup>b</sup>
	10	67.21 <sup>b</sup>	84.82 <sup>b</sup>
	15	66.60 <sup>b</sup>	80.73 <sup>ab</sup>
NaOH	2	64.60 <sup>b</sup>	85.38 <sup>b</sup>
	4	67.10 <sup>b</sup>	80.13 <sup>ab</sup>
	6	62.67 <sup>ab</sup>	76.70 <sup>a</sup>
Urea	10	61.39 <sup>ab</sup>	82.59 <sup>ab</sup>
	20	62.13 <sup>ab</sup>	81.92 <sup>ab</sup>
	30	58.61 <sup>a</sup>	80.07 <sup>ab</sup>

*CPA = Cocoa pod ash. Different superscript at the same column shows the statistically different at  $p < 0.05$*

In vitro Gb, estimate OMD and ME of treated CPs showed in table 3. Ensilage and application of urea up to level U2 gave a significant improvement of Gb, but at U3 level Gb drop dramatically. Estimate OMD and ME showed that urea treatment is better than silage.

**Table 3: Gas production, estimated of digestibility and Metabolizable energy**

Treatments	Doses g/kg	Gb	OMD	ME
		ml/200 mg DM	%	MJ/kg DM
Fresh		9.41 <sup>c</sup>	32.61 <sup>ab</sup>	3.40 <sup>b</sup>
Silage	0	11.51 <sup>d</sup>	33.04 <sup>ab</sup>	3.64 <sup>b</sup>
CPA	5	8.85 <sup>bc</sup>	31.70 <sup>ab</sup>	3.20 <sup>ab</sup>
	10	8.17 <sup>b</sup>	31.75 <sup>ab</sup>	3.13 <sup>ab</sup>
	15	9.28 <sup>bc</sup>	33.47 <sup>b</sup>	3.26 <sup>ab</sup>
NaOH	2	9.68 <sup>c</sup>	31.98 <sup>ab</sup>	3.28 <sup>b</sup>
	4	8.05 <sup>b</sup>	31.57 <sup>a</sup>	3.01 <sup>a</sup>
	6	9.08b <sup>c</sup>	33.17 <sup>ab</sup>	3.14 <sup>ab</sup>
Urea	10	12.07 <sup>d</sup>	38.43 <sup>c</sup>	4.45 <sup>c</sup>
	20	12.99 <sup>d</sup>	42.13 <sup>d</sup>	5.08 <sup>d</sup>
	30	4.86 <sup>a</sup>	42.07 <sup>d</sup>	5.06 <sup>d</sup>

## Discussion

The failure of CPA in improving the cocoa pod quality was caused by the moister content of media was too high. The soluble ash like potassium (source of alkali process) leached downward to the bottom layers and caused the bottom layer was over dosage. Although NaOH theoretically stronger than urea but NaOH application quite limited. Application of 2 – 6 g NaOH granulate per kg cocoa pod was difficult to reach the homogenous medium. As a result there was some part of the cocoa pod media over dosage, and other is under dosage. To reach the homogenous medium for both treatments, the better application is needed. Dilution of NaOH in water and drying the cocoa pods before the CPA and NaOH applications may be one of solution.

On urea application, the NH<sub>3</sub> as product of ureolysis, in water gave middle alkali (NH<sub>4</sub>OH) reaction and diluted the mineral-SiO<sub>2</sub> linkage. The alkali processed also strengthened the cellulose and hemicelluloses-lignin linkage. The facts were shown by reducing the ash and crude fibre contents. The availability NH<sub>3</sub> and carbon source on urea treatments promote the micro-organisms population growth which was indicated by the gas production (fermentation activities).

Response of Gb on application of urea (Figure 1) followed equation of  $-0.0016U^3 + 0.0489U^2 - 0.2757U + 11.51$ , where U is the amount of urea/kg fresh matter CPs. The Gb peaks at U about 17 g urea per kg fresh matter CPs. At this level, urea application gave the highest organic fermentation. Application of higher urea decrease fermentation activity. At level U3 the Gb extremely low and at this level urea become toxic to the rumen microbe.

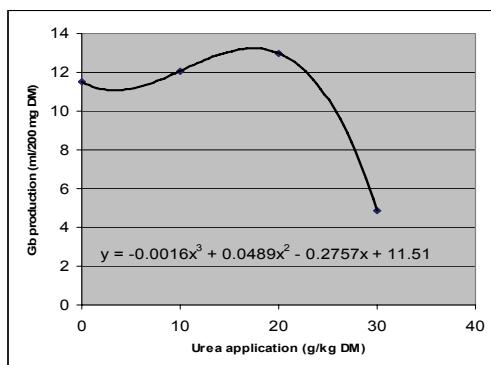


Figure 1: Gas production as response to urea application

Estimation of DOM and ME by Gb and proximate composition as recommended by Menke et al. (1979) can not be applied for urea treated cocoa pods. The Gb production is irrelevant to the DOM and ME especially for the U3 treatment. The increasing of CP value of urea treated cocoa pod gave overestimate values of DOM and ME. Therefore other protein value is necessary to estimate DOM and ME of the urea treated feed.

The improvement of CPs quality was also shown by the silage treatment. Even the improvement of the OMD and ME estimate were not as high as urea treatment (because there is no improvement in CP content), the treatment is recommended as an alternative in shortage availability of urea. The incubation of cocoa pod for 2 weeks without alkali agent is better then fed the animal with fresh CPs.

## Conclusion

Urea is the most suitable alkali source for CPs quality improvement in this study. Application of 17g urea per kg fresh matter CPs is recommended. On local absence of the alkali source, the improvement quality of cocoa pod with silage treatment is also recommended.

## References

Chenost, M.; Kayouli, C., 1997: Roughage Utilization in Warm Climates. Animal Production and Health Paper 135. FAO, Rome.

Flachowsky, G.; Kamra, D.N; Zadrazil, F., 1999: Cereal Straws as Animal Feed – Possibilities and Limitation. J. Appl. Anim. Res. 16: 105 – 118.

Menke, K.H.; Raab, L.; Salewski, A.; Steingass, H.; Fritz, D.; Schneider, W., 1979: The Estimation Of The Digestibility And Metabolizable Energy Content Of Ruminant Feedstuffs From The Gas Production When They Are Incubated With Rumen Liquor. J. Agric. Sci. 93, 217 – 222.

Naumann, C.; Bassler, R., 1997: Vdlufa-Methodenbuch Band Iii, Die Chemische Untersuchung Von Futtermitteln. 3rd Ed. Vdlufa-Verlag, Darmstadt, Germany.

Schiere, J.B.; Ibrahim, M.N.M., 1989: (Eds.) Feeding Of Urea Ammonia Treated Rice Straw. A Compilation of

Miscellaneous Reports Produced by the Straw Utilization Project (Sri-Lanka). Pudoc, Wageningen, Pp. 125.

Sundstøl, F.; Mgheni, D.M.; Pederson, I., 1993: Recent Finding On Upgrading Of The Feeding Value Of Straw By Chemical And Biological Methods. Proceedings of the International Conference on Increasing Livestock Production through Utilization of Local Resources. 8 – 12 October 1993. Beijing – China.

Van Soest, P.J.; Robertson, J.B., 1979: Systems of Analysis for Evaluating Fibrous Feeds. In: Standardization of Analytical Methodology for Feeds. Proceedings of a Workshop Held in Ottawa, Canada, 12-14 March, 1979.

# Utilization of Methanol Extracted Of Moringa And Mulberry Leaves To Evaluate Energy and Protein Balance Of Nile Tilapia

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## Abstract

*Fish ration should have high protein content. Source of feed protein usually comes from animal such as fish meal and waste of fishery industries. The price of animal protein like fish meal is quite expensive and that ingredient of feed has competitive problem with human food. Plant protein like legume leaves or other forage can be used for covering protein requirement of herbivore fish, but they contain high secondary compounds. These compounds may be removed by methanol extraction of the plant material, e.g. for moringa. The present study was carried out to evaluate energy balance of diet containing extracted moringa leaves and mulberry leaf meal each as 30 % protein replacement for fish meal in diets for Nile Tilapia. Three diets were designated as control diet prepared with fishmeal (C), diet 1 contain methanol extracted moringa (D-1) and diet 2 contained mulberry leaves (D-2). Fifteen Nile Tilapia were randomly kept in a 5 L capacity individual respiration chamber in which the oxygen consumption of each fish could be measured continuously (Focken et al., 1994). Prior to the experiment fish were fasted for two days in order to measure standard metabolic rate (SMR), routine metabolic rate (RMR) and spontaneous activity (SSA). After those measurements, fish were divided into three groups and fed with the test diets C, D-1 and D-2 at around 10-g feed per MBW (kg<sup>0.8</sup>) using automatic feeders. Fish were weighed individually every week and the oxygen consumption continuously measured for gain information on the energy expenditure (EE). At the end of the eight week, fish were sacrificed and analyzed for energy retention (ER). Feed analyses were conducted to evaluate gross energy intake (GEI), while energy metabolism (ME) was calculated from EE plus ER. The data were subjected to ANOVA and statistical comparisons between the feeding groups were made using the Duncan's Multiple Range Test. Results showed that the average values of SMR, RMR and SSA were 49, 67 and 105 mg.kg<sup>0.8</sup>.h<sup>-1</sup>, respectively. Energy intake for the control group was lower than for the other groups, while final body weight in group D-1 was the highest. The ratio EE and ME from GEI (%) were similar for groups, while ER (g) for group D-2 is the highest. It was concluded that methanol extracted moringa leaves and mulberry leaves are quite palatable and could replace 30 % of protein fish meal in diets for Nile Tilapia.*

**Keywords:** Moringa, Mulberry, legume tree, SMR, RMR, SSA

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## Introduction

Fish ration should have high protein content. Source of feed protein usually comes from animal such as fish meal and waste of fishery industries. The price of animal protein like fish meal is quite expensive and that ingredient has competitive problem with human food. Plant protein like legume leaves can be used for covering protein requirement of herbivore fish, but in tropical legumes they contain high secondary compounds, which have a side effect to the user. The other alternative forage which also content high protein is *Moringa oleifera* Lam and mulberry (*Morus* sp.) leaves.

*Moringa oleifera* is tree which grows throughout most of the tropics and has several industrial and medicinal uses (Becker and Makkar, 1999). They are not legumes and also not a gramineae; some people call it “The Miracle Tree”. They has multifunction such as human food, water purification, medicinal products and animal and fish feed (Becker and Makkar, 1999; Foidl *et al.*, 2001). In Indonesia, in such area like Bali, Madura, Nort Sumatra and South Sulawesi island, people eat those leaves and especially for lactating mother. While in India, Nicaragua and Niger there are a lot of *Moringa oleifera* plantation and uses for multi purposes. It was reported that replacement of 20 and 30 % of the total dietary protein with freeze-dried *Moringa oleifera* leaf meal for Nile tilapia had decreasing of growth performance caused of the relatively high secondary compounds like total phenolics, saponin and phytic acid, as well as NDF and ADF ( Richter *et al.*, 2003). Afuang *et al.* (2003) reported that methanol-extracted residues and methanol extracts of moringa leaf meal had no significant effect on the growth performance compared with control diet in Nile tilapia and so far it was concluded that those diets reduced the plasma and muscle cholesterol. The nutritional and energy content of extracted and unextracted moringa leaves are 43.50 and 25.10; 1.40 and 5.40 ; 47.40 and 21.90; 16.30 and 14.10 %; 17.70 and 18.70 MJ/kg for CP, CL, NDF, ADF and GE, respectively ( Gupta *et al.*, 1989). Makkar and Becker (1996) reported that anti nutritional components of whole and extracted moringa leaves which is important information for animal feed are glucosinolates, saponin, total phenols, tannins and cyanogenic glycosides in pars of moringa plant.

There are a lot of Mulberry species. Ekastuti *et al.* (1996) reported the nutrient content of five kinds of mulberry leaves such as *Morus cathayana*, *M. nigra*, *M. canva*, *M. multicaulis* and *M. alba* from Indonesia in different cutting stage which have 15.71 – 22.59; 3.70 –

615 ; 8 – 16.8 % and 3.5 – 4.6 Kal/ kg for protein, lipid, fiber and gross energy respectively.. Those leaves also content vitamin A, where *M. cathayana* has the highest one compared to the other (5671 and 5736 mg%, in young and old leaves respectively. The proximate analysis of sun-dried mulberry leaves also reported by Phiny *et al.*, (2003) which were contained (%DM) 20 and 22 % of CP and CL respectively, and around 75 % of DM digestibility.

There is no information regarding the utilisation of methanol extracted of moringa and raw mulberry leaf meal to report the digestibility and energy balance of Nile tilapia. Therefore, the present study was carried out to evaluate of digestibility and energy balance of diet containing extracts moringa and mulberry leaf meal as 30 percent protein replacement for fish meal in diets for Nile tilapia.

## **Material and methods**

### *Diet formulation*

*Moringa oleifera* and mulberry leaves were obtained from Indonesia with treated oven-dried 40 ° C before transportation to Germany. On receipt at Hohenheim University, they were finaly ground in a laboratory mill and from the moringa stock; sample was extracted with 90 % methanol using a Soxhlet apparatus for 48 h. The leaf extracts were separated from the residues through filterred using a filter paper and the residues were freeze-dried soon after air-drying and all material above stored at freezer until analysis and feed formulation. Prior to feed formulation, the proximate composition and amino acid analysis of methanol extracted moringa and morus leaves were determined, while wheat meal and fish meal were analyzed by previous researcher (Richter *et al.*, 2003).

Three diets, were designated as control diet ©, diet 1 (D-1) and diet 2 (D-2), were used in this experiment. The control diet was prepared with fishmeal as the primary source of protein by mixing with various ingredients as shown in Table 1. Diet 1 and diet 2 were designated to replace 30 % of fishmeal-derived dietary protein in diet using methanol extracted residues of moringa leaf and raw mulberry leaf meal respectively.

Table 1. Chemical composition and anti-nutrient content of ingredients (g % DM)

	Wheat meal	Fish meal	Me-OH moringa	Morus
DM	87.70	90.90	91.30	90.30
CP	14.00	69.90	37.40	29.58
CL	1.50	8.10	3.80	1.40
CF	1.70	-	7.60	20.12
Ash	1.63	19.47	12.52	9.64
NDF	-	-	42.28	20.34
ADF	-	-	22.01	15.59
Met + Cys	0.80	3.19	1.42	0.65
Val	0.91	4.40	1.97	1.47
Isoleu	0.68	3.63	1.75	1.22
Leu	1.14	6.16	3.61	2.46
Phe +Tyr	1.60	5.39	4.05	1.53
His	0.46	1.65	1.31	0.92
Lys	0.57	5.61	2.08	1.84
Thre	0.91	5.50	1.97	1.39
Arg	0.57	3.41	2.85	1.86
Tryp	0.23	0.88	0.77	0.54
Saponin	-	-	3.04	1.72
Tannin	-	-	trace	0.46
Phytic acid	-	-	trace	2.91
Total phenolic	-	-	trace	1.04

### *Experimental Design*

A group of nile tilapia (11 – 24 g) fingerlings were used in fish laboratoriu of Departement of Aquaculture System and Animal Nutrition, Institute for Animal Production in the Tropics and Subtropics, Hohenheim Universty. There were two batches of experiment where experiment 1. was for evaluation of energy balance using respiratory boxes while experiment 2. was for evaluation of digestibility of diets containing methanol extracted moringa and raw mulberry leaf meal. At the beginning of the experiment, three fish of the same population were killed and frozen for the determination of initial body composition and the rest of fish were fed at level of maintenance requirement according to body weight.

### *Experiment 1: In Respiration chamber*

Fifteen nile tilapia were randomly kept in the 5 L capacity individual respiration boxes in which the oxygen consumption of each fish could be measured continously (Focken *et al.*, 1994). The boxes were

illuminated 12 h on and off and water temperature was kept at 28,2 °C. During acclimatisation (3 days) in the experimental set up, the fish received maintenance level of feed. In the prior of experiment fish were fasted for two days according to measure standard metabolic rate. The standard rate of O<sub>2</sub> consumption (SMR) was determined when the fish had grown used to the respiration box approximately after 24 h, then the VO<sub>2</sub> values collected at the start of the experiments were tested for SMR which was attained when low metabolic rates had been measured constantly over 60 min. Routine VO<sub>2</sub> (RMR) is the O<sub>2</sub> consumption of a fasting fish over 24 hours including the VO<sub>2</sub> resulting from spontaneous activity, the highest VO<sub>2</sub> values recorded in the first 48 hours of experiment is the spontaneous activity (SSA) (Becker *et al.*, 1986).

#### *Experiment 2: In Aquaria*

Another fifteen nile tilapia fingerlings (11 – 22 g) were kept in the 40-l of individual aquaria for acclimatisation. The aquaria were integrated into a recirculatory system at 26.6 °C. They were divided into three groups of 5 fish each randomly and fed at around maintenance level (3 g/ kg<sup>0.8</sup>/day) with three kind of diet containing approximately 35 % protein, 7 % lipid, 11 % ash and a gross energy content of 19 kJ g<sup>-1</sup> dry matter. After adaptation three fish of the same average body weight were killed and analysed for initial body composition. During the experimental period, the fish were fed at 10 g feed per metabolic body weight (kg<sup>0.8</sup>) per day in four equal installments using an automatic feeder. Fish were weighed individually every week. At the end of eight week of the experiment, fish were weighed, sacrificed and analyzed for whole body composition. Prior to the chemical analyses, both the initial and experimental groups of fish were autoclaved at 120 °C for 30 min, homogenised, refrozen and freeze dried.

Table 2. Formulation of experimental diets (g %DM)

	C	D-1	D-2
Wheat meal	45	31	25
Fish meal	41	29	29
Me-OH moringa	0	28	0
Morus	0	0	37
Mineral mix	2	2	2
Vitamin mix	2	2	2
Sunflower oil	4	4	4
Alpha cellulose	5	3	0
Ti O <sub>2</sub>	1	1	1
TOTAL	100	100	100

Table 3. Proximate and amino acid composition, of experimental diets and amino acid requirements of Nile tilapia (% DM)

requirement	C	D-1	D-2	tilapia's
Dry matter	94.30	97.5	93.02	-
Crude protein	36.72	35.97	35.95	-
Crude lipid	7.90	6.98	6.37	-
Ash	10.95	11.89	11.74	-
Gross energy (kJ/g)	19.16	18.80	19.80	-
NDF	20.38	31.32	24.38	-
ADF	8.0	10.74	6.99	-
Met + Cys	1.67	1.57	1.36	0.90
Val	2.21	2.11	2.05	0.80
Isoleu	1.79	1.75	1.67	0.90
Leu	3.04	3.15	2.98	1.0
Phe +Tyr	2.93	3.19	2.53	1.60
His	0.88	0.99	0.93	0.50
Lys	2.55	2.39	2.45	1.40
Thr	2.66	2.43	2.34	1.20
Arg	1.65	1.96	1.82	1.10
Tryp	0.46	0.54	0.51	0.30

#### *Biochemical analysis*

The proximate analysis of diet ingredients, diet and whole bodies of fish (CP, CL, and ash) were based on the procedures of the AOAC

(1990) standard methods. Dry matter was measured by drying to a constant weight at 105 °C and gross energy by bomb calorimetry (IKA C 7000) with benzoic acid standard. Fiber constituents such as neutral detergent (NDF) and acid detergent (ADF) fibers of methanol extracted moringa and raw morus leaves were determined according to the procedure described by Van Soest *et al.* (1991). An automated amino acid analyser was used to determine the amino acid composition of feed ingredient. The total phenolics and tannins were determined by the spectrophotometric methods described by Makkar *et al.* (1993). Phytic acid estimation was carried out by the modified photometric procedure of Vaintraub and Lapteva (1988) and the total saponin content was determined by the method of Hiai *et al.* (1976).

#### *Calculation and statistical analysis*

All calculations were performed for each fish individually. Growth performance was assessed in terms of the Body Weight Gain (BWG) which calculated by subtracting final and initial body weight, Feed Conversion Ratio (FCR) was calculated as live weight gain/feed consumption (dry matter), and Metabolic Growth Rate (MGR) as live weight gain (g)/average metabolic live weight ( $\text{kg}^{0.8}$ )/day. The Average Metabolic Rate was calculated as mg oxygen consumed  $\text{kg}^{0.8}/\text{h}$  on a weekly basis. The Standard Metabolic Rate (SMR) was taken as the lowest metabolic rate sustained for 2 h by the undisturbed fish that had been fasted for the preceding 24 h (Ultsch *et al.* 1980). This calculation was done using the oxygen consumption values recorded on the day during which the fish were starved, before experimental feeding started. Oxygen uptake (g)  $\times$  14.85 (kJ/g) gave the energy expenditure (EE) during the whole experiment (Huisman 1976) and the energy apparently metabolised (ME) was calculated by subtracting energy retention and energy expenditure of carcass (ER) from the gross energy of the feed consumed. Diet nutrient utilization was analysed in terms of Feed Intake (FI), Protein Production Value (PPV, %) was calculated as protein gain  $\times$  100/feed protein. Protein Efficiency Ratio (PER) was calculated as live weight gain (g)/ protein fed (g).

The data were subjected to ANOVA and statistical comparisons between the feeding groups were made using the Duncan's Multiple Range Test (Statistica for Windows, release 5.1 H, 97 edition). The significance of observed differences was tested at  $p < 0.05$ . the values presented in the text are Mean  $\pm$  Standard Deviation.

## Results

### Respiration chamber experiment

All data of the experimental chamber was shown in Table 4. where from the statistic analysis there were no significance difference between treatment.

The data of Nile Tilapia's SMR were same with reported before which were around  $44.4 \text{ mg/kg}^{0.8}/\text{d}$  (Becker, 1990). The scope for spontaneous activity was a good measure of the energy available to the fish for body tissue synthesis. The RMR and SSA values in this experiment were also similar with reported before. Tilapia zillii has RMR and SSA were around  $64.4 \text{ mg/kg}^{0.8}/\text{d}$  and  $111.2 \text{ mg/kg}^{0.8}/\text{d}$ , respectively.

Table 4. Metabolic rates of the experimental fish

	Control	D-1	D-2
Standard metabolic rate (SMR):			
- mg/kg <sup>0.8</sup> /h	42.10	49.43	55.81
Routine metabolic rate (RMR):			
- fasting (mg/kg <sup>0.8</sup> /h)	62.65	73.43	67.44
Scope for spontaneous activity (SSA)			
- fasting (mg/kg <sup>0.8</sup> /h)	105.24	108.42	103.6

### 2. Aquaria experiment

The chemical composition of the experimental fish was in Table 5. There was significant difference in crude lipid of control and treatments where were fishes fed with moringa and morus had low lipid ( $P<0,05$ ). Total energy of body in D-2 was significantly lower than other treatments ( $P<0,05$ ).

Table 5. Initial and final chemical composition of the experimental fish

	Initial Mean	Control Mean	D-1 Mean	D-2 Mean
DM (% of fresh)	20.24	27.99	28.42	25.62
CA (% DM)	10.71	16.34	16.25	17.52
CP (% of DM)	66.05	56.66	57.26	58.29
CL (% of DM)	12.38	22.79 <sup>a</sup>	19.97 <sup>b</sup>	18.21 <sup>b</sup>
GE (kJ g-1)	15.57	21.17 <sup>a</sup>	21.06 <sup>a</sup>	19.95 <sup>b</sup>

The calculated average values of nutrient utilization, FCR and biological value of diet are presented in Table 6. The BW gain value in D-1 was significantly higher than those of the control and D-2. This is because of high the initial BW and nutrient intake in that treatment. Palatability of the ration in D-1 was good for tilapia. As the consequences utilization of nutrient in D-1 was better than D-2, but not significantly different with control. The highest FCR value was done in D-2 (P<0.05).

Table 6. Growth performance and nutrient utilization of the experiment fish

SD	C Mean	SD	D-1 Mean	SD	D-2 Mean
Initial BW (g)	16.5b		18.9a		18.0a
Final BW (g)	29.3b		38.9a		31.4b
BW Gain (g)	12.8b		20.0a		13.4b
Feed offered (g)	16.3b		21.1a		19.5a
Protein intake (g)	5.9b		7.7a		7.0ab
Lipid intake (g)	1.3b		1.7a		1.4b
Energy intake (kJ)	311.5b		396.5a		387.1a
FCR	1.0b		1.1b		1.5a
PER	2.7a		2.6a		1.9b
PPV (%)	46.6b		55.3a		45.8b
ER (%)	40.9a		44.6a		35.1b

The complete energy budget of the fish in the different experimental groups was set up in Table 7. There were differences in

the GE intake and energy retention (% GE) between the treatments. In control treatment was showed efficiency in energy utilization with low energy intake but high percent of energy retention, while in the D-2 treatment was in opposite where high energy intake produced low energy retention. The best energy utilization was happened in D-1 treatment.

Table 7. Energy budget of fish in different experimental group

	C		D-1		D-2	
	Mean	SD	Mean	SD	Mean	SD
Initial GE of carcass (kJ)	39.7		39.7		39.7	
Final GE of carcass (kJ)	166.6b		185.0a			
GE intake (kJ)	311.5b		396.5a			
EE (% GEI)	25.5		24.9			
ER (% GEI)	40.9a		44.6a			
AUE (% GEI)	31.4		38.9			
ME (% GEI)	66,4		69,5			
	59,4					

## Discussion

All the experimental fish consumed the feed provided completely and there were no mortality of fish during the experiment. Diet containing moringa and mulberry leaves were palatable for fish. Energy budget in tilapia fed with extracted moringa and morus have same heat production which around 25 %, while the energy retained in morus group was lower than another treatments. Protein efficiency ratio (PER) in D2 showed the worst. Mulberry leaves which still contained secondary compound (saponin, phytic and tannin) was affected to the performance. The value of PER in diet control and D1 were same with reported before (Francis *et al.*, 2002), while the value of PPV (45 – 55%) was higher in this experiment compared to fish fed with quillaja saponin which had PPV around 32- 36 % (Francis *et al.*, 2002). The

physiological effect of saponins on fish has been controversial, some authors reporting positive and others negatives influences. Roy *et al.* (1990) reported that saponin depressed blood parameters such as hematocrit, haemoglobin and red blood cell in several species of fish. On the other hand Francis *et al.* (2002) reported that supplementation of quillaja saponin in diet carp resulted high oxygen consumption and indicating higher metabolic activity.

There is usually an increase in body fat and energy content with increasing body size in fish fed maximum rations (Cui *et al.*, 1996). In present study, body size had significant effects on the content of fat and energy of Nile tilapia. This is because of all rations has good palatability and quality. The ration was prepared as NRC requirement for Nile tilapia. Even though diet with mulberry leaves was not as efficient as other treatment. Theoretically the proportions of food energy in growing animals would be allocated to various organ target in the body and resulted by the size. Shouqi Xie *et al.* (1997) reported that in mature sexual fish resulted in reduced growth rate caused by the decrease in relative food intake.

Utilization of fresh moringa in fish diet was reported by Richter (2003) with very bad performance of the Nile tilapia. There is no information about morus on diet fish. Ekastuti (2006) reported utilization of kinds of morus leaves with different moisture levels on silk worm production. In this experiment showed that morus leaves without extraction resulted good performance in Nile tilapia although was not same as moringa methanol extracted.

## Conclusion

1. Moringa extracted and morus leaf meal could substitute 30 % of fish meal in Tilapia diet.
2. Energy budget of Tilapia fed with extracted moringa have same pattern compare to Control, while morus treatment was different.
3. The energy expenditure and energy retention were around 25 % and 40 %, respectively.

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## References

Afuang, W., P. Siddhuraju and K. Becker. 2003. Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research* 34:1147-115

Becker K. and Lev Fishelson, 1986. Standard and routine metabolic rate, critical oxygen tension and spontaneous scope for activity of tilapias. The first Asian Fisheries Forum. Asian Fisheries Society, Manila, Philipines. P 623-628

Cui, Y., Hung, S.S.O., Zhu, X., 1996. Effect of ration and body size on the energy budget of juvenile white sturgeon. *J. Fish Biol.* 49, 863-876

David Sklan, tal Prag and Ingrid Lupatsch. 2004. Apparent digestibility coefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus* x *Oreochromis aureus* (Teleostei). *Aquaculture research*, 35: 358-364

Ekastuti, D.R., D.A. Astuti, R. Wdjajakusuma and D. Sastradipradja. 1996. Rearing silkworm (*Bombyx Mori*) with artificial diets as an effort to promote the quantity and quality of national rawsilk production. Research Report, Research Institute of IPB, Bogor, Indonesia. June 1996.

Focken U., K. Becker and P. Lawrence, 1997. A note on the effects of L-carnitine on the energy metabolism of individually reared carp, *Cyprinus carpio* L. *Aquaculture Nutrition* 3:261-264

Francis, G., H.P.S. Makkar and K. Becker. 2001. Effect of Quillaja saponins on growth, metabolism, egg production and muscle cholesterol in individually reared Nile tilapia (*Oreochromis niloticus* L.). *Comparative Biochemistry and Physiology part C* 129:105-114

Francis, G., H.P.S. Makkar and K. Becker. 2002. Effects of cyclic and regular feeding ofa Quillaja saponin supplemented diet on growth and metabolism of common carp (*Cyprinus carpio L.*). Fish Physiology and Biochemistry 24: 343-350

Francis, G., H.P.S. Makkar and K. Becker. 2002. Dietary supplementation with Quillaja saponin mixture improves growth performance and metabolic efficiency in common carp (*Cyprinus carpio L.*). Aquaculture 203:311-320

Gupta,K. G.K. Barat, D.S. Wagle and H.K.L. Chawla, 1989. Nutrient contents and antinutritional factors in conventional and non conventional leafy vegetables. Food Chemistry. 31: 105-116

Jobling, M. 1994. Fish Bioenergetic. Chapman and Hall. New York.

Makkar,H.P.S. and K. Becker. 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Anim. Feed Sci. and Tech. 63 : 211-228

Nikolaus Foidl, H.P.S. Makkar and K. Becker. 2001. The potential of *moringa oleifera* for agricultural and industrial uses. In: The Miracle Tree (Ed: Lowell J. Fuglie). CTA, Postbus 380, 6700 AJ Wageningen, the Netherlands

Richter, N., Perumal Siddhuraju, K. Becker. 2003. Evaluation of nutritional quality of *moringa* (*Moringa oleifera Lam*) leaves as an alternative protein source for nile tilapia (*Oreochromis niloticus L.*). Aquaculture 217. Pp 599-611

Roy, P.K., Munshi, J.D., Dutta, H.M., 1990. Effect of saponin extracts on morpho-histology and respiratory physiology of an air breathing fish. J. Freshwater Biol. 2, 135-145

Shouqi Xie, Yibo Cui, Yunxia Yang and Jiankang Liu. 1997. Energy budget of Nile tilapia (*Oreochromis niloticus*) in relation to ration size. Aquaculture 154: 57-68

Shouqi Xie, Yibo Cui, Yunxia Yang and Jiankang Liu. 1997. The effect of body size on growth and energy budget of Nile tilapia, (*Oreochromis niloticus*). Aquaculture 157 pp. 25-34

Zarkadas, C. G., Z. Yu and V.D. Burrows. 1995. Protein quality of three new Canadian-developed naked oat cultivar using amino acid compositional data. Journal of Agric. And Food Chem. 43: 415-421

## **A study of carcass and meat chemical composition of babirusa (*Babirousa babyrussa celebensis* Deniger)**

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### **Abstract**

*Babirusa is an endemic germplasm animal in Sulawesi. Existence population of this animal in the rain forest habitat of Sulawesi decreases continuously every year due to illegal logging and firing forest destruction, illegal hunting, animal predators and diseases. Therefore, conservation strategy of babirusa must be applied to increase its population and to enrich its potential product varieties for local people consumption need. To support this purpose, scientific information of carcass percentage meat chemical composition of babirusa is important to be studied. The objective of this study is to evaluate carcass percentage, backfat thickness, protein and fat content in meat of babirusa. The total numbers of babirusa used in this research were ten adult animals (five males and five females). Research was conducted using observation method to collect primary and secondary data. Primary data was observed directly from samples based on laboratory analysis. Secondary data was found from information of hunters, people around habitat location and institution involving this research. Variables used in this study were habitat, feeding, morphological characteristics of animals, backfat thickness, meat chemical composition and carcass percentage. The results of this study showed that environmental factor (animal activities in habitat) was a factor affecting carcass quality (mainly meat fat contents and backfat thickness) of babirusa.*

**Key words:** babirusa, carcass, chemical composition

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## Introduction

Babirusa is a natural potential and a genetic endemic germplasm animal in Sulawesi, needed to be bred. Babirusa population in rain forest of North Sulawesi was increasing abundantly during several decades ago, but its population was decreasing gradually in recent years. This decreasing population was due to its habitat destruction, illegal hunting, predators and diseases.

Babirusa was hunted by people from Minahasa region to utilize its meat as a food product. Its meat has a specific delicious taste and low fat content. In addition, babirusa has high meat production, indicated by the fact that male babirusa reached life weight of 100 kg with body length of 100 cm (from snout to buttock part of animal body) and shoulder height of about 70 cm, while physical size of female animals were smaller than those of male animals (Veevers and Carter, 1978).

This wild animal had a potential to be bred as a domestic animals producing meat. Its meat was more superior compared with meat of wild pig and meat of Timor deer, especially in term of nutrient content, viscosity, texture and flavor (Rekswardojo, 1995).

Carcass quality was affected by two main factors, namely genetic factor and environmental factor (Lawrie, 1979; Soeparno, 1994). Genetic factor affected carcass quality as indicated by the fact that pig (Duroc strain) had characteristic trend to the deposit intramuscular fat (Soeparno, 1994). Carcass of wild animal at tropical area was significantly different with that of domestic animal. A significant difference was fat content which was lower in wild animals (Williamson and Payne, 1993).

Backfat thickness was a parameter to determine carcass quality, because two third of total fat in carcass were subcutaneous fat; consequently, measurement of backfat thickness became a good indicator for determination of carcass quality (Blakely and Bade, 1991). Backfat thickness was also a factor to be included in determination of total meat which was produced in unit of carcass (Soeparno, 1994).

Meat was classified in physical and chemical composition. Main chemical compositions of meat were water, protein, fat, minerals, vitamin and carbohydrate. Meat consisted of 75 % water content (ranging from 68% to 80%); 19% protein content (ranging from 16% to 22%); 3.5% soluble non protein substance content and 2.5% fat content (ranging from 1.5% to 13.0%). Protein was main nutrient component

materials in meat. High nutrient contents in meat were due to a completed and balanced essential amino acids (Soeparno, 1994).

One parameter to be used for evaluation of animal product was carcass percentage. Carcass percentage was a comparison between carcass weight and life weight of animal multiplied by a hundred percent (Blakely and Bade, 1991). Carcas percentages of pig with 50 to 60 kg life weight were less than 70%, while carcass percentages of pig with life weight of more than 60 kg were 70 to 80% (Whittemore, 1993).

For the purpose of breeding, conservation, increasing population and enrichment of productive variation of babirusa, the main scientific information of carcass percentage and chemical meat composition of babirusa was needed to be evaluated to explore this research.

## **Materials and Methods**

### **Research Materials**

This research was conducted at district of Paguyaman, Province Gorontalo, during 90 days. Ten adults babirusa (5 males and 5 females) were used in this study. Equipment used in this study were length gauge (cm/m), weight gauge (g/kg), refrigerator, cool box, plastic bags, backfat thickness gauge (mm), thermometer, knife, photo kamera,usw.

### **Source of Data:**

Suvey method according to Singarimbun and Effendi (1995) was applied in this study. Primary and secondary data were used in this study. Primary data were obtained directly by observation and by result of laboratory analysis. Secondary data were obtained from information of hunter, local community around forest and other institution related to this study.

### **Research Variables:**

Variables observed in this study consisted of animal habitat (including forest status, climate, topography, flora and founa, and water sources), feeding (including feed ingredient, nutrient content and nutrient sources), characteristic and morphological aspects of animal (including body length, head length, body height, foot length and chest circumference), backfat thickness (including the average of measuring

point of first chest bone, point of the last chest bone, point of the last back bone of animal), meat chemical composition (including protein and fat content) and carcass percentage (comparison of carcass weight and live body weight multiplied by one hundred). Carcass was body parts of animal without blood, foot, head, digestive tract and other internal organs (Soeparno, 1994).

### **Procedures of Physic and Carcass Measurements**

- a. Live body weight was measured by weighing animals using hang scale tool with capacity of 110 kg.
- b. Physical performances were observed by measuring animal body length, chest circumference, foot length, head length and body height.

### **Procedures of Freezing Meat and Laboratory Analysis**

Meat was taken from parts of thigh, leg, loin, jaw, chest, belly, shoulder of slaughtered animal and filled those parts into plastic bag, then sealed them to avoid dehydration process. Meat in plastic bags were stored into ice box with temperatures ranging from -4°C to 1°C during maximum time period of 24 hours during transportation, then stored them again in to freezer with temperature of - 18°C (Soeparno, 1994).

Laboratory analysis of meat was done to evaluate protein content by Kjeldahl method and fat content by Soxhlet method.

### **Data Analysis**

Data were statistically analyzed by calculating mean and percentage of standard deviation (Steel and Torrrie, 1993).

## **Results and Discussions**

### **General Habitat Condition**

Survey location was focused at Adudu rain forest, district of Paguyaman, Province of Gorontalo. Topographical condition was flat to slope up to 300 m from sea level, and other location was slope up to 600 to 2095 m from sea level (located at Boliohuto Mountain). Average of rainfall precipitation at Paguyaman district was 2560 mm per year, where heavy rainfalls were occurring from November to February and sunny dry seasons were generally occurring from July to September with temperatures ranging from 22°C to 32°C (Clayton, 1991).

In this area, at least nine species of wild mammal animals were inhabiting around rain forest, such as anoa (*Bubalus depressicornis*), tarsier (*Tarsius spectrum*), deer (*Cerous timorensis*), tupai (*Prosciurillus murimus*, *P. leucomus*, *P. rubriventer*) and macaque (*Macaca hecki*). Bird species observed in this area were red-knobbed hornbill (*Rhyticeros cassidix*), small hornbill (*Penelopides exarhatus*), kerust (*Trichoglossus ornatus*), Sulawesi lorry (*Prioniturus sp*), pigeon (*Ducula sp*), black dove (*Chalcophaps indica*) and collared kingfisher (*Halcyon chloris*).

Some endemic wild animals such as megapoda (*Megapodius cummingii*), wild hens (*Gallus gallus*), python snake (*Python reticulatus*), green snake (*Trimerurus sp*) and monitor lizard (*Varanus salvador*) were obtained around rain forest base.

Specific habitat of babirusa was found along the edges of river and water filling places grown by wild grasses. As reported by Macdonald (1993) that some of babirusa was also found at places closed to beach area. Veevers and Carter (1978) reported that babirusa dominated on the wet and muddy places. Adudu rain forests were dominated by luxuriant vegetation of some plants such as beringin (*Ficus sp.*), nantu trees (*Palaquim obtusfolium*), rao trees (*Dracontomelon rao*) and other trees. Base areas of rain forest were grown by scrub plants such as small palms, rattan, wild bananas and grasses causing high humid condition of land surfaces.

Adudu rain forest had salt water sources used by babirusa to drink and to remain in the muddy places. These salt water places were unique phenomenon due to being far away from beach areas. There were three salt water places in Adudu rain forest. First salt water place was called salt water Adudu, second place was called salt water Abati, and third place was called salt water Lontolo. Salt water Adudu was the biggest area with a size of 60 m length and 30 m width. Temperature was around 50 °C at water source center. This place was also covered by black muddy soil at the thickness of about 20 cm, and ground soil consisted of sandy and rocky bases. These areas were openend to get sun shines. Around these places were covered by vegetation mainly consisted of *Pandanus sp* and grasses. Therefore, theses places became center place of some animal species to drink, such as anoa, deer and wild pigs. At the location of obseved areas, there were two big rivers (Nantu river and Adudu river), and some water streams becoming place centers for most animals to drink. There were also some fresh water area centers as place of babirusa. Generally, babirusa were grassing and looking for feeding at sunny days (Nowak, 1991), as well as took rest

under branches of trees. Macdonald (1993) reported that babirusa made its nesting place under stone caves on the base of mountain. Habitat destruction due to activities of local community to take rattan and illegal logging of some enterprises could decrease population of babirusa.

### Feeding

Generally, babirusa fed fruits, leaves and roots of wild trees, wood worms and carrion (Muttaqin *et al.*, 1993) as presented in Table 1.

**Table 1. Identified Feeding of Babirusa at its Habitat**

No.	Identified Feeding	Local Name	Scientific Name
1	Fruit	Jongi	<i>Dilenia sp</i>
2	Fruit	Aren	<i>Arenga pinnata</i>
3	Fruit	Pangi	<i>Pangium edule</i>
4	Fruit	Nantu	<i>Dracontomelon mangiferum</i>
5	Fruit	-	<i>Caryota mitis</i>
6	Fruit	Rao	<i>Dracontomelon rao</i>
7	Fruit	Nangka Hutan	<i>Carpus sp.</i>
8	Leaves	Pandan	<i>Pandanus sp.</i>
9	Fruit	-	<i>Coorsidenron pinatua</i>
10	Grasses	-	-
11	Tuber	Ubu Talas	<i>Colocasia sp.</i>
12	Carrion	-	-
13	Wood worm	-	-

Based on their feeding ingredients, babirusa was classified into omnivore animal (Table 1). Macdonald (1993) reported that babirusa had cannibalism characteristic to feed their offspring and even to feed offspring of other animal species. Two main feeding ingredients of babirusa at observed location were dominated by *Pangium edule* and *Arenga pinnata*. The chemical compositions of those feeding ingredients were presented in Table 2.

Mineral needs of babirusa around their habitat could be reached through their feed and through natural salt water of Adudu, Lantolo and Abati. Muttaqin *et al.* (1993) reported that babirusa always took contact directly with muddy soil. Babirusa dug muddy soil using its snout during they were remaining in muddy places. Parakkasi (1990) reported that babirusa was utilizing soil as source of minerals. Macro

and micro minerals were helping growth metabolism process and formation of body tissue, and then affecting carcass quality.

**Table 2. Fruit Chemical Composition of Pangi (*Pangium edule*) and Aren (*Arenga Pinnata*).**

Nutrients	Kinds of Fruit		
	<i>Pangium edule</i>		<i>Arenga pinnata</i>
	Meat	Seed	Seed
Protein (%)	13.34	23.64	0.75
Fat (%)	0.92	37.98	-
Carbohydrate (%)	52.15	25.92	3.12
Fiber Crude (%)	3.64	3.35	1.06

Feeding availability around animal habitat could be affected by several factors such as seasons and feed competition among animals. In addition, habitat destruction such as loss of animal feed sources due to limited areas could cause loss of nutrient and size of areas providing feed sources (Alikodra, 1990).

### **Characteristic and Morphology of Babirusa**

Babirusa was included into *Artiodactyla* animals, class of Suidae, and sub class of *Babyrousinae*, giving the same characteristics with pigs. External characteristics of babirusa were including grey hair color, but giving white color on parts of chest, belly and feet. Other characteristic were indicated by wrinkle skin around belly, legs and neck. Babirusa had gold brown hair with length of 0.2-0.5 cm and also had short tail. Back part of its body had the shape of curve with behind part of that was higher than front part of the body.

Male adult babirusa had a unique characteristic completed with a pair of tusk bending toward forehead look like a pair of horns. On the lower jaw, there was also a pair of small tusk. The older the animal, the longer a pair of tusks. Female babirusa did not have a pair of tusks on the snout, but it had small tusk growing on the lower and upper jaws.

The other characteristic of female babirusa was the fact that it had two pairs of teats; therefore it usually had offspring of 1-2 heads. Babirusa had sexual maturity of 5-10 months old and had natural long life of about 24 years old. Estrus cycle period of babirusa was ranging from 28 to 42 days and female adult babirusa showed again a natural estrus cycle after three months post partum. Estrus period of babirusa

was ranging from 2 to 3 days. Nonestrus adult females did not have sexual libido of mating with adult males (Macdonald, 1993). The gestation period of babirusa was about 158 days (Reksowardojo, 1995).

Measurement result of physical performance of male and female babirusa compared with those of wild pigs were presented in Tabel 3.

**Tabel 3. Average Measurement of Physical Performance of Babirusa and Wild Pigs**

Physical Measurements	Babirusa		Wild Pigs	
	Male	Female	Male	Female
Life weight (kg)	36.6 ± 12.1	28.7 ± 4.3	30.1 ± 12.1	20.4 ± 2.7
Body length (cm)	106.4 ± 9.8	96.8 ± 8.4	98.8 ± 6.3	90.0 ± 5.3
Body height (cm)	64.8 ± 7.2	58.4 ± 4.7	57.9 ± 0.7	51.5 ± 3.1
Chest circumference (cm)	78.7 ± 12.8	73.5 ± 7.6	73.7 ± 1.9	66.0 ± 5.6
Front leg height (cm)	59.2 ± 8.5	59.0 ± 4.8	47.7 ± 4.4	42.4 ± 2.5
Behind leg height (cm)	63.0 ± 8.9	54.4 ± 5.5	50.3 ± .5	44.9 ± 2.6
Head length (cm)	32.6 ± 2.0	30.3 ± 2.0	31.4 ± 0.8	20.4 ± 0.6

Measurement results showed that physical sizes of babirusa were higher than those of wild pigs. In addition, physical sizes of male babirusa and male wild pigs were higher than those of female babirusa and female wild pigs.

#### **Backfat Thickness and Meat Fat Content**

Average backfat thickness of babirusa was 7.57 mm and meat fat content was 1.27%. Pig of Yorkshire strain and Landrace strain had backfat thickness of 34.3 mm and 38.9 mm, respectively (Zainal, 1995). Meat fat contents of domestic pigs were 5-15% (Whittemore, 1993). Therefore, backfat thickness and meat fat content of babirusa were generally lower than those of domestic pigs. Averages of backfat thickness, meat nutrient content and carcass percentage of babirusa were presented in Table 4.

**Table 4. Averages of Backfat Thicknes, Meat Nutrient Content and Carcass Percentage of Babirusa**

Backfat Thickness	Meat Nutrient Content		Carcass Percetage (%)
	Protein (%)	Fat (%)	
7.57±3.8	20.27±0.1	1.27±0.2	69.74±2.8

Soeparno (1994) reported that carcarss was affected by genetic and environmental factors. Animal activity arround its habitat was an environmental factor affecting component of animal body, especially fat content deposited in their body. Muttaqin *et al.* (1993) reported that one babirusa needed cross land areas of about 300 ha, and most of its activities were looking for feeding, remaining on the muddy place, making nest, swimming accros river, fighting, avoiding risk and breeding (Proyek Pengembangan Alam dan Amdal, 1990; Nowak, 1991; Macdonald, 1993; Patry *et al.*, 1995).

Low backfat thickness and meat fat content in babirusa were due to daily high activities of muscle around their habitat. Guyton (1981) reported that high contraction of muscle needed energy of ATP (Adenosine Triphosphate) supplied by consumption of nutrien diet. During formation and supply of ATP for muscle contraction, more energy of nutrient was utilized for metabolism to be converted into heat energy. Guyton (1992) reported that about 55 % energy of nutrient were converted into heat during ATP formation. Therefore, fat deposition (backfat and meat fat) in babirusa was low becouse other parts of nutrient diet were used for muscle activities. Parakkasi (1990) reported that consumption of nutrient diet exceeding needs of basic life maintenance and production caused the excess of energy stored into fat tissues. Low deposition of fat in the body was also affected by genetic factor (Soeparno, 1994). Carcass composition of each animal differed from one to another animal and had its own characteristics; for example, pig of Duroc strain tended to deposit intramuscular fat. Rekswardojo (1995) reported that meat crude fat content of babirusa maintained at animal garden park was very low (1.6%).

### **Meat Protein content**

The average meat protein content of babirusa was 20.27% (Tabel 4). High activity of babirusa around their habitat affected meat protein content. Guyton (1981) reported that repeated strong muscle activities caused increasing size of muscle. These muscle tissues obtained additional total number of myofibril, other nutriens and materials intermetabolism. These additional total numbers of myofibril in muscle tissues were equal with those of protein. Meat protein content of babirusa was comparable with that of pigs of about 20-25% (Whittemore, 1993). Rekswardojo (1995) reported that meat protein content of babirusa maintained in animal garden Park was 21.0%.

## **Carcass Percentage**

Carcass was body weight of slaughtered animal without head, blood, legs, digestible tracts and other internal organs. Factors determining carcass value were carcass weight, meat produced and meat quality (Soeparno, 1994). Average carcass percentage of babi rusa in this study was 69.74%. This carcass percentage was the same with that of domestic pigs which was about 70% (Whittemore, 1993).

## **Conclusion**

Based on results of this study, it can be concluded that environment (including animal activities around their habitat) was a factor affecting carcass quality of babirusa (mainly meat fat content and backfat thickness).

## **References**

Alikodra, H.S. 1990. Pengelolaan Satwa Liar. Departemen Pendidikan Kebudayaan. Dierktorat Jenderal Pendidikan Tinggi . PAU IPB, Bogor

Blakely, J dan D.H. Bade. 1991. Ilmu Peternakan. Terjemahan Bambang., S. Gajah Mada. University Press, Jogyakarta

Clayton, L. 1991. Survey Reporty Wild Life in Pagoyaman Area, Regent of Gorontalo. Wildlife Conservation Unit. University of Oxford

Guyton, A.C. 1981. Fisiologi Kedokteran I. Terjemahan Dharma A.P. Lukmanto. Penerbit Buku Kedokteran EGC, Jakarta

Guyton, A.C. 1992. Fisiologi Kedokteran II. Terjemahan Dharma A.P. Lukmanto. Penerbit Buku Kedokteran EGC, Jakarta

Lawrie, R.A. 1979. Ilmu daging. Terjemahan Parakkasi, A. Universitas. Nottingham, Cambrige

Macdonald, A. 1993. The babirusa (*Babirousa babirussa*) IUCN/SSC. Hippo Specialist Group

Muttaqin, L., D.W. Sinaga, A.H. Mustari, L.M. Clayton dan S.H. Lagurusu. 1993. Survei Kehidupan Babirusa di Kabupaten Gorontalo Sulawesi Uatara. Departemen Kehutanan Republik Indonesia

Nowak, R.M. 1991. Mammals of the World. The Johns Hopkin University Press, Baltimore and London

Parakkasi, A. 1990. Ilmu Guzi dan Makanan Ternak Monogastrik. Penerbit. Angkasa Bandung

Patry, M., K. Levis and A.A Macdonald. 1995. Group Structure and Behavior of Babirusa (*Babyrousa babyrussa*) in Northern Sulawesi. Australian Journal Zoology 43,643-55

Proyek Pengembangan Kawasan Pelestarian Alam dan Amdal. 1990. Pedoman Pengenalan Jenis Satwa yang Dilindungi Undang-Undang. Buku I Klas Mamalia.

Reksowardojo, D.J. 1995. Studi Kemampuan dan Produksi Babirusa (*Babyrousa babyrussa celebensis* Deniger) melalui Upaya Budidaya. Program Pascasarjana IPB, Bogor

Singarimbun, M dan S. Effendi. 1995. Metode Penelitian Survei. LP3ES.Jakarta

Soeparno, 1994. Ilmu dan Teknologi Daging. Gajah Mada University Press, Jogyakarta

Steel, R.G.D dan J.H. Torrie. 1993. Prinsip dan Prosedur Statistika: Suatu Pendekatan Biometrik. Terjemahan Bambang S. PT. Gramdia, Jakarta

Veevers, W dan Carter. 1978. Mamalia Darat Indonesia. Penerbit PT. Intermasa, Jakarta

Williamson, G dan W.J.A. Payne. 1993. Pengantar Peternakan di Daerah Tropis. Terjemahan Darmadja, S.G.M.D. Gajah Mada Press University. Jogyakarta

Whittemore, C. 1993. The Science and Practice of Pig Production. Longman Scientific and Technical, England

Zainal, G.M. 1995. Pengaruh Penggunaan Ampas Kelapa Sebagai Bahan Subtitusi Ransum dan Air Kelapa Pengganti Air Minum Terhadap Kualitas Karkas Babi Periode Finisher. Fakultas Peternakan. Universitas Sam Ratulangi, Manado

## **Nutritional Properties of Three Different Origins Of Indonesian Jatropha (*Jatropha Curcas*) Meal For Ruminant**

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### **Abstract**

*An experiment to explore the nutrition properties of Indonesian Jatropha curcus meal for ruminant has been conducted. Three different origins of Indonesian jatropha (Lampung, Kebumen and Lombok) have been investigated for their nutritional values such as chemical compositions, amino acid profiles, fermentability and in vitro digestibility. Anti nutritional and toxic compounds are also determined. It was found that chemical compositions and amino acid profiles of the Jatropha meal vary according to the origin of Jatropha. The jatropha meal from Lampung had the highest CP (% DM) content (42.58%) compared with jatropha meal from another region (Kebumen 37.93%, Lombok 32.94%). The CP (%Solid Non Fat) content of jatropha meal from Lampung (58.59%) was higher than CP content of soybean meal (50.71%). The jatropha meal from three different regions in Indonesia showed a significant difference ( $p<0.01$ ) in IVMD and IVOMD, which is the jatropha meal from Lampung (60.23%) and Kebumen (60.73%) was higher than from Lombok (49.8%). Toxic and anti-nutritional factors that studied in this research are curcin, phorbolester and phytate. The phytate and curcin level was highest in jatropha meal from Lampung. But the level of phorbolester was not detectable in this meal. The contents of toxic and anti-nutritional factors did not affect on digestibility and ruminal fermentation products.*

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## Introduction

*Jatropha curcas* L. is known as a prospective renewable energy source which to be developed in Indonesia. *Jatropha curcas* represent annual crop which hold up dryness so that able to grow and expand better in marginal land as in region of East Indonesia (Hambali, 2006).

The oil from the kernels of *Jatropha curcas* came through processing phase. Jatropha seed extraction can be conducted by using simple expeller machine. Jatropha meal is the residue from seed processing. The extraction will obtained 30-40% oil and 60% jatropha meal. The residue in the form of jatropha meal has potential as animal feed. However, due to the toxicity further research need to be conducted to have the basic information from jatropha meal.

The objective of this research were to get the basic information of jatropha meal include the information of the nutrition, anti nutrition and toxic factors which is the main constraint of *Jatropha curcas*, and to study the characteristics of jatropha meal as animal feed especially for ruminant.

## Materials and Methods

### Sample Preparation

The jatropha seeds used were from three different regions from Indonesia (Lampung, Kebumen and Lombok). The seed were dehulled, dried and ground manually. The kernel was defatted using manual hydraulic press machine. The defatted and ground kernel is referred to as the meal and used for next analysis. Soybean meal was used for some analysis as comparison.

### Chemical Analysis

Chemical compositions of jatropha meal were determined according to AOAC (1980) procedure. The amino acids analysis was conducted using HPLC, while the gross energy was measured using Bomb Calorimeter. The concentration of phorbolester of jatropha meal was carried out by de procedures describes by Makkar *et al.* (1997), while lectin/cursin was carried out by heamagglutination assay (Aregheore *et al.* 1998). The concentration of phytate was determined using spectrophotometry procedure.

### ***In Vitro Digestibility***

The *in vitro* dry matter and organic matter digestibility were determined using the two-step method according to Tilley and Terry (1963).

### ***Gas Production***

Gas production of jatropha meal was measured using Hohenheim Gas Method (Close and Menke, 1986). Samples (230 mg) were put in shyring glasses and then added 30 ml suspension of rumen fluid and McDougall buffer. The samples were incubated in water bath at 39°C for 0, 4, 8, 12, 16, 20 and 24 hours. Gas production can be calculated using the formula:

$$\text{Gas production (ml/200mg DM, 24h)} = [(Gb24-Gb0)*200]/DM \text{ samples}$$

where:

- Gb 0 = gas production 0 hour
- Gb 24 = gas production 24 hour
- DM = dry matter

### ***NH<sub>3</sub> Concentration and VFA Total Production Analysis***

The analysis of ammonia concentration conducted with Conway Microdiffusion method and VFA total production analysis conducted with Steam Distillation technique (General Laboratory Procedure, 1966).

### ***Statistical Analysis***

The data were subjected to analysis of variances using the general linear model procedure of the SPSS package program. The differences between means were tested using the Duncan's Test.

## **Results and Discussion**

### ***Nutritional Composition***

The nutritional composition and gross energy of jatropha meal are shown in Table 1. There was some variation in the contents of lipid, crude protein, crude fiber and gross energy. Generally the nutritional composition (%DM) of jatropha meals in this study was relatively higher than the soybean meal, except for the crude protein content.

However, if the crude protein content was calculated in percentage from Solid Non Fat (% SNF), the crude protein content of jatropha meal was higher than soybean meal.

**Table 1. Chemical composition of jatropha meal from three different origins in Indonesia**

Compositions	Origin			Soybean meal
	Lampung	Kebumen	Lombok	
Dry Matter (%)	93.19	93.24	94.10	89.51
Ash (% DM)	7.31	7.01	6.78	6.33
Lipid (% DM)	20.52	22.38	29.62	2.45
Crude Protein (% DM)	42.58	37.93	32.94	44.15
Crude Protein (% SNF)	58.59	53.53	51.09	50.71
Crude Fiber (% DM)	13.82	12.97	6.58	3.20
Gross Energy (cal/g)	5062	4713	4915	-

### ***Amino acid composition***

The amino acid composition of jatropha meal is shown in Table 2. The amino acid content of jatropha meal from three different regions was similar. A comparison between the amino acid composition of jatropha meal and soybean meal reveal a different pattern for all essential and non-essential amino acids, except methionine and arginine. The level of this amino acid was higher in the jatropha meal.

### ***Toxic and Anti-nutritional factors***

Toxic and anti-nutritional factors are the main constraint of utilization of jatropha meal as animal feed. Cursin is a toxic protein compound of jatropha. However, recent report showed that cursin is not major toxic principle in *Jatropha curcas* meal (Aderibigbe *et al.* 1997). The toxicity of cursin can be reduced by heat treatment. The level of cursin of jatropha from three regions varied from 0.67 – 0.72%.

Phorbolester is a major toxic of *Jatropha curcas* (Makkar and Becker, 1997). The phorbolester was found in high level in the sample from Lombok. Generally, the phorbolester content of jatropha from Indonesia was relatively lower (0.99 – 1.33 mg/g) than toxic varieties from Cape Verde, Nicaragua, and Nigeria (more than 2 mg/g sample) (Makkar *et al.*, 1998).

Phytate is the anti-nutritional factor that being observed in this research. Hyatt level have been implicated in decreasing protein digestibility by forming complexes and also by interacting with enzymes such as try sin and pepsin (Reddy and Pierson, 1994). The hydrate level in jatropha meal was high (6.65%-7.39%), these values

are much higher than that of soybean meal (Table 3). The highest phytate level was found in the sample from Lampung.

**Table 2. Amino acid composition of jatropha meal from three different origins in Indonesia**

Amino Acids (%)	Origin			Soybean meal
	Lampung	Kebumen	Lombok	
<i>Essential</i>				
Methionine	0.18	0.44	0.29	0.37
Lysine	1.04	1.19	1.11	2.22
Valine	1.30	1.56	1.44	1.91
Isoleusine	1.12	1.33	1.24	1.86
Leusine	1.94	2.33	2.21	2.99
Tyrosine	0.78	1.02	0.91	1.45
Phenilalanine	1.31	1.53	1.44	1.97
Histidine	0.72	0.84	0.78	0.96
Threonine	1.03	1.24	1.12	1.53
<i>Non-essential</i>				
Aspartic acid	2.93	3.54	3.16	4.92
Serine	1.42	1.75	1.59	2.08
Glutamic acid	5.46	6.77	6.07	9.22
Glycine	1.23	1.50	1.41	1.57
Alanine	1.39	1.66	1.58	1.74
Arginine	3.57	4.39	4.07	3.04
Total amino acids	25.42	31.09	28.42	37.83

**Table 3. Toxic and anti-nutritional factors of jatropha meal from three different origins in Indonesia**

Toxic and Anti-Nutrition	Origin			Soybean meal
	Lampung	Kebumen	Lombok	
Curcin (%)	0.72	0.70	0.67	-
Phorbolester (mg/g seed)	Nd	0.99	1.33	-
Phytate (%)	7.39	6.65	7.00	-

### ***Digestibility***

The *in vitro* dry matter and organic matter digestibility (IVDMD and IVOMD) is shown in Table 4. There was significant differences ( $p<0.01$ ) in the IVDMD and IVOMD of jatropha meal from different regions. The digestibility of jatropha meal from Lampung and Kebumen was significantly higher than from Lombok. The digestibility of jatropha meal was also similar with soybean meal.

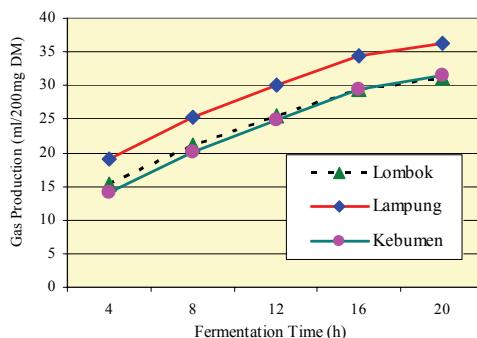
**Table 4. DMIVD and OMIVD of jatropha meal from three different origins in Indonesia**

Digestibility	Origin			Soybean meal
	Lampung	Kebumen	Lombok	
IVDMD (%)	60.23±3.90 <sup>a</sup>	60.73±4.89 <sup>a</sup>	49.48±5.96 <sup>b</sup>	63.54±4.38
IVOMD (%)	59.63±2.93 <sup>a</sup>	60.82±3.63 <sup>a</sup>	48.57±5.35 <sup>b</sup>	63.64±4.61

\* The different superscript in the same rows showed a significant differences ( $p<0.01$ )

### **Gas Production**

The ruminal fermentation of carbohydrates, proteins and lipids will produce gas. The gas production measurement is to estimate digestion process in the rumen. The fermentable feed will be degraded faster and produced higher gas. Jatropha meal from Lampung showed the highest gas production. The highest increase is presented at the 0-8-h of incubation (Figure 1).



**Figure 1.** Ruminal gas production of jatropha meal

### **Ammonia and VFA's concentration**

Ammonia is the main nitrogen source of the amino acid synthesis for rumen microbes. Those metabolism process reveal that protein for ruminants is depend on the rumen protein synthesis process. The product of protein hydrolysis was degraded to produce the ammonia. VFA's is the main product from the carbohydrate fermentation in ruminants. Polysaccharide is being hydrolyze to get the monosaccharide and then to get the volatile fatty acids,  $\text{CO}_2$  and  $\text{H}_2$ .

The ammonia concentration and VFA total production is shown in Table 5. The mean of ammonia concentration and VFA total

production showed non-significant differences. It reveals that the anti-nutritional and toxic of jatropha meal did not affect the microbes' activity in fermentation of carbohydrates and proteins.

**Table 5. Ammonia and total VFA's concentration of jatropha meal from three different origins in Indonesia**

Concentration	Origin			Soybean meal
	Lampung	Kebumen	Lombok	
Ammonia (mM)	14.58±4.99	15.70±4.64	11.24±1.84	12.65±9.50
VFA (mM)	131.39±8.34	147.56±25.15	148.02±12.63	159.42±6.7 7

### Conclusions

The physical and chemical characteristics of jatropha meal in this research showed some variation. It can be concluded from this research that the contents of toxic and anti-nutritional factors did not affect on digestibility and ruminal fermentation products. Nevertheless, it can't be concluded that the jatropha meal is safe to be given as animal feed.

### References

Aderibigbe, A. O., Johnson, C. O. L. E., Makkar, H. P. S. and Becker, K., 1997. Chemical composition and effect of heat on organic matter and nitrogen degradability and some anti-nutritional components of jatropha meal. *Anim. Feed Sci. Technol.* 67, 223-243.

AOAC, 1980. *Official Methods of Analysis*. 13th Edition. Association of Official Agricultural Chemists. Washington DC.

Aregheore, E. M., Makkar, H. P. S. and Becker, K., 1998. Assessment of lectin activity in a toxic and a non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. *J. Sci. Food Agric.* 77, 349-352.

Close, W. and K. H. Menke. 1986. *Selected Topics in Animal Nutrition*. The Institute of Animal Nutrition, University of Hohenheim. Germany.

General Laboratory Procedure. 1966. Department of Dairy Science. University of Wisconsin Madison.

Hambali, E. 2006. Jarak Pagar, Tanaman Penghasil Biodiesel. Cetakan kedua. Penebar Swadaya. Jakarta.

Makkar, H. P. S. and Becker, K., 1997. *Jatropha curcas* toxicity: identification of toxic principle(s). In: 5th International symposium on poisonous plants. May 19 -23, 1997, San Angelo, Texas, USA.

Makkar, H.P.S., Aderibigbe, A.O., Becker, K. 1998. Comparative evaluation of non-toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chemical. 62 (2): 207-215.

Reddy, N.R. and Pierson, M.D. 1994. Reduction in anti-nutritional and toxic components in plant foods by fermentation. Food Research International. 27:281-290.

Tilley, J.M.A. and R.A. Terry. 1963. A two stage technique for *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18:104-111.

# **The Effect of Bay Leaves Infusum (Syzygium polyanthum (Wight)) on anti inflammation in White Rat Sprague-Dawley**

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## **Introduction**

*Medicinal plant has long been used in Indonesia to maintain health since a long time, based on empirical evidence. People only notice the effect of eating medicinal plant instead of explanation on how the medicinal plant works. Individual medicines in developing countries vary considerably in quality. Herbs used for medicinal purposes are “crude drugs”. These unprocessed herb plant or plant parts are dried and used in whole or cut forms. Herbs are prepared as teas, sometimes as capsules for internal use and as poultices for external use. Usually medicines are developed from plants. Much modern day medicine is directly or indirectly derived from plant sources. Therefore it would not be correct to conclude that plants offer no further potential for the treatment or cure of the major diseases. World wide, the botanical pharmacopoeias contain tens of thousands of plants used for medicinal purposes (Darma in Ietje et al., 2000). In Indonesia there have been many vegetables and herbs that have been widely used as traditional medicinal plants since ancient times. There are 40.000 species of medicinal plants in the world, and 30.000 species among them grow in Indonesia, including 90% species which have been identified to have medicinal effect, 74% species cultivars are found wild in the forests, thick forests, fields and garden plantations, 26% species the remaining or equivalent with 940 species which have known, but only 17% which have been exploited as basic material of traditional medicines commercially (BAPPENAS, 1996; Hamid et al., 1991).*

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*Exploiting traditional medicines from all the times tends to increase because of the desire of society itself to re-use natural materials to improve the health degree (BAPPENAS, 1996). Before being used and marketed widely, traditional medicines have to be tested about the quality and the safety of the medicine. Some researches that have to be done are as follows: filter tests to know the compound of the plant; toxicity test to know the safety when it is consumed for medication, experimental test and clinic test to ascertain the pharmacology effect, security and clinic benefit (Goth 1984, 2003).*

### **Objectives**

The aim of this research is to know anti-inflammation effective in white rat of Spraguay-Dawley male type from bay leaves infusum (*Syzygium polyanthum* (Wight) Walp at various concentration.

### **Materials and Methods**

This research was conducted using facilities at the Laboratory of Toxicology, Department of Physiology and Pharmacology - FKH IPB. In January - February 2005.

#### ***Experimental animals***

Thirty growing male Spraguay-Dawley of similar body weight is about 180-200 gram divided into 6 groups. The animals were obtained from the Research of Veterinary (BALITVET) Bogor. The animals were housed in the cages in a room to adapt to laboratory conditions.

#### ***Bay leaves infusum***

Bay leaves obtained from the wild growing tree in front of house. The process of bay leaves was done according to infusum method which is fresh bay leaves being collected, withered and dried-up process in the oven at temperature of 50°C for 3 days. Dry bay leaves then being milled and become powder. Bay leaves infusum obtained by dissolving 100 g bay leaves powder with 900 ml aquadest. This solution was heated and stirred at temperature 90°C, 15 minutes, then filtered.

#### ***Procedure***

During the experiment, the animals were offered their respective diets ad libitum.

### **Anti-Inflammation Test**

Thirty growing male Spraguay-Dawley rats of similar body weight were used divided into 6 groups, five animals each. The back right foot dipped into phenol 50% during 3 seconds to get inflammation then attempt materials given orally. As shown in the table 1 below:

**Table 1. Treatment of the rats**

<b>Treatment group</b>	<b>Given attempt materials peroral</b>
1. Normal Control	Is not continued with any treatment
2. Aquadest Control	Given 2 ml aquadest
3. Na-diklofenak Control	Given 2 ml Na-diklofenak
4. Bay leaves 100%	Given 2 ml Bay leaves the concentration of 100%
5. Bay leaves 200%	Given 2 ml Bay leaves the concentration of 200%
6. Bay leaves 400%	Given 2 ml Bay leaves the concentration of 400%

To see the effect of giving treatment, perception and measurement of swelling done at 0 minute, 15, 30, and at the first hour, 2, 4, 24 after the back right foot dipping into phenol 50%. The data was analyses using ANOVA at reliable level of 95% and level of  $\alpha$ , 0,05. If the result showed in the real difference it will be continued to Duncan test.

### **Result and Discussion**

Through the Duncan test, obtained the results which showed control group tends to have the first alphabet letter. This matter indicates that the control group's inflammation effect tends to be high. While group of given Bay leaves tends to have the end alphabet letter, which indicates that giving of Bay leaves can reduce inflammation effect.

In 30 minutes inflammation effect of normal control group real differ ( $P<0,05$ ) had the highest inflammation effect compared to the other treatment group. At the first hour, inflammation effect of Bay leaves group 200% real differ ( $P<0,05$ ), with the lowest inflammation effect compared to the group control and the Bay leaves group 100%, but it is not real differ ( $P<0,05$ ) than the Bay leaves group 400%. At the second hour inflammation effect of Bay leaves group 200% real differ ( $P<0,05$ ), with the lowest inflammation effect compared to the other treatment group. At 24 hours inflammation effect of aquadest control group and Bay leaves group 100% showing the real differ result ( $P<0,05$ ), with higher inflammation effect than the other treatment group.

At the first hour, normal control group have the highest inflammation effect, whereas Bay leaves group 200% have the lowest inflammation effect. At the second hours to 24 hours, aquadest control group have the highest inflammation effect, whereas Bay leaves group 200% have the lowest inflammation effect. At second hours inflammation effect of phenol control group is equal to aquadest control group. At fourth, all treatment groups have shown degradation of inflammation effect.

Complexity of biological system and the impact generated by correct medicine dose to organism caused the interaction with specific receptor or network as according to expected impact to be influenced by physics-chemistry medicine and tieing medicine-receptor bound. Physics-chemistry characteristic of medicine are dissolving, partition coefficient, ionization degree, and surface activity (Korolkovas, 1970).

Dissolving has important role at biological action some medicines. Dissolving had a close of relationship with absorption. Because tendency of biological action in medicine will depend on the absorption degree. Dissolving of medicine at water is called hydrophilia or lyphofobia, whereas dissolving at grease is called lyphilia or hydrophobia (Korolkovas, 1970).

Biological activity from some medicines can be connected by the partition coefficient at polar and nonpolar dissolver. Partition coefficient is comparison of concentration at grease phase with liquid phase when a molecule goes to concentration balance (Goldstein *et. al.*, 1969).

Improvement of ionization will result the dissolving medicine in water and declining the dissolving medicine in grease. This matter affect to absorption and past of medicine pass the resistance and grease membrane, and concentration of medicine at rich network of grease (Korolkovas, 1970).

Some molecules of medicine can decline of surface tension by concentration the molecule at surface of liquid, so that the biological activity happened (Korolkovas, 1970).

### ***Normal Control***

In zero minute to first hour, fast improvement of highest inflammation happened but compared than the other treatment group, phenol is soluble water and make skin irritation (Jenkins, *et al.*, 1957). The toxic effect a substance adsorbed through skin usually have the character grease dissolve, can destroy permeability of skin so that cause the skin and layer dermis of skin consisting of connective and capillary

tissue become very permeable to all molecules, both for dissolve in grease and dissolve in water (Goldstein *et. al.*, 1969). Without the existence of treatment delay the phenol activity, this toxic substance easy to have diffusion to skin network, and cause inflammation at the flatten skin.

At the first hour to 24 hours, the fast degradation of inflammation happened. The happening of fast degradation and inflammation effect has designate detoxification and degradation of phenol toxicity. The molecule tends to have non polar character and dissolve in grease including phenol can easily be adsorbed by tubular cell of kidney and metabolism become the dissolve molecule in water so that easy to be excreted. Metabolism process enlarging the molecule more polar, dissolve in water, cause detoxification and declining of toxicity (Ariens *et. al.*, 1964).

### ***Aquadest Control***

At zero minute to first hour, the fast improvement of inflammation happened although the effect is lower compared with the inflammation at normal control, but higher rather than the inflammation of the other treatment group. Molecule of water tends to easily ionize (Kimber *et. al.*, 1956). Water had diffusion through membrane pores with a few amount at cell membrane. Most of cell membranes tend to permeable to non ion molecule which able to dissolve in grease and less permeable to ion molecule form (Goth, 1984). When the distribution of water was reach for the network of inhibition phenol, the smaller part phenol dissolve in the water. This matter may cause the resistance of inflammation because the dissolve ness phenol in water does not distribution quickly, because the phenol cannot dissolve in grease but ion with water so that have to pass the pores at cell membrane in a few number.

At second hours to 24 hours, the fast degradation of inflammation happened. Fast degradation of inflammation at aquadest control group is the slowest compared with the other treatment group. The molecule which dissolve in water and form ion from the most medicines cannot enter the cell quickly (Goldstein *et. al.*, 1969). The phenol which dissolve in water decelerate in the distribution in body, so that detoxification process and degradation of phenol toxicity through metabolism process rather pursued. This matter showed that the inflammation effect at aquadest control group remain higher than the other treatment groups.

### ***Sodium Diclofenak Control***

At zero minute to second hours, the fast improvement of inflammation happened. Anti-inflammation medicine non steroid work by pursuing synthesis and release prostaglandin.. Pharmacology effect cover reduction of inflammation symptom, downhill of body temperature, lessen to feel the pain without eliminating awareness, and lessen full scale platelet (Goth, 1984).

At second minutes to 24 hours, the fast degradation of inflammation happened. Degradation rate and inflammation effect show that the medicine had pharmacology effect. Effect of medicine is influenced by medicine-receptor bound and characteristic of physico-chemistry between medicine and receptor which is important functionally for mortal (Goldstein *et. al.*, 1969).

### ***Bay leaves 100%, 200%, and 400%***

At Bay leaves groups 100%, 200%, 400%, fast improvement of inflammation happened in 0 minute to second hours, and fast degradation of inflammation happened in second hours to 24 hours.

At Bay leaves groups 100%, the fast improvement of inflammation happened in 0 minute to second hours. At this concentration, volume content irrigate as dissolver tends more compared with the Bay leaves group concentration 200% and Bay leaves group 400%, so that the inflammation effect still up of the other Bay leaves group. (Kimber *et. al.*, 1956; Goth, 1984). This matter affect at slower of distribution, also difficult of active component to bundle with specific receptor, because have to penetrate the water coat becomes dissolver (Goldstein *et. al.*, 1969).

All perception time of inflammation effect of Bay leaves group 200% showed that lower effect than the other treatment group inflammation effect. Bay leaves 200% have highest effective as anti-inflammation, so that this concentration is the best and correct dose used in medication. Interaction between medicines with receptor happened when correct concentration water phase is achieved, so that medicine must be able to penetrate on receptor to reach the effective concentration. Correct dose needed to the stimulus a total of potential molecules, is so that yielded the maximal effect (Ariens, 1964).

At Bay leaves group 400% have the best effectiveness of anti-inflammation compared with Bay leaves 100%, but it is not better than the Bay leaves 200%. To produce the effect, medicine-receptor must be able to produce enough stimulants. The decreasing contents of water will cause the decreasing composition of molecule medicine to receptor,

therefore the maximal effect may occurred, when all receptor filled by molecule medicine (Ariens, 1964).

Complex total medicine-receptor which formed at Bay leaves group 100%, 200%, and 400% influenced by the injected dose (dose administration), relation between doses medicine and effective concentration of water phase (transfer of medicine), and the ability of medicine to bind with receptor (affinity) (Ariens, 1964). Transferred medicine and affinity determined the medicine potency, which is the medicine dose that will generate the effect (Devoted *et. al.*, 1995). Potency medicines pursuant to the most effective of anti-inflammation are Bay leaves group 200%, Bay leaves group 400%, and Bay leaves group 100%.

Fast degradation and inflammation effect at the Bay leaves group 100%, 200%, and 400% distinguishing the medicine have been given the effect pharmacology. Medicines effect, influenced by binding the medicine-receptor and the nature of physics-chemistry between medicines and receptor, is important functionally for mortal (Goldstein *et al.*, 1969). Jenkins *et. al.*, (1957) say that sour ester of acetate, compound phytochemistry which consist in Bay leaves, used by plant to detoxication alcohol and fenol. Bisset *et. al.*, (1991) say that flavonoid, compound phytochemistry, which also consist in Bay leaves, have the activity anti-inflammation by pursuing the release histamin. This matter caused the Bay leaves group 100%, 200%, and 400% tending to have lower inflammation effect compared to the control group.

## **Conclusions and Suggestions**

### ***Conclusions***

- Bay leaves is medicine plant with effect LD<sub>50</sub> practical not toxic
- Infusum of Bay leaves can be function as anti-inflammation
- Bay leaves 200% is most effective concentration as anti-inflammation

### ***Suggestions***

To examine chronic toxicity and prepared the histophatologic to know the usage effect of infusum Bay leaves on along term.

## References

Areins EJ. 1964. Nolucular Pharmacology. The Mode of Biologically Active Compounds. Volume I. New York and London: Acaemic press.

BAPPENAS. 1996. Strategi Perkembangan Agribisnis dan Agroindustri Tanaman Obat secara Terpadu dalam Prosiding: Forum Konsultasi Strategi dan Koordinasi Pengembangan Agroindustri Tanaman Obat, Bogor: Badan Penelitian dan Pengembangan Pertanian Balai Penelitian Tanaman Obat dan Rempah

Bisset NG, Houghton PJ, Hylands PJ., 1991. Some Trends in Medicinal Plant Research in the Medicinal Plant Industry. Edited by ROB Wijesekara. 1991. Florida: CRC Press.

GoldsteinA, Aronow L, Kalman S., 1969. Principles of Drug Action. The Basis of Pharmacology. New York: Harper International Edition.

Goth A, 1984. Medical pharmacology. Principles and Concepts.11 edition. St. Louis Toronto: The C.V. Mosby Company.

Hamid A, Hadad EA dan Rostianan O., 1991. Upaya Pelestarian Tumbuhan Obat di BALITRO dalam Prosiding Pelestarian Pemanfaatan Tumbuhan Obat dari Hutan Tropis Indonesia, Bogor: Institut Pertanian Bogor.

Ietje W, Chakareda S, Meulen UT., 2002. Effect of Curcuma (*Curcuma xanthorrhiza*) on Lipid Metabolism in Rabbits, Journal of the Science of food and Agriculture

Jenkins GL, Hartung WH, Hamlin KE, Data JB., 1957. The chemistry of Organic Medicinal Products. New York: John Wilwy and Sons Inc. London: Chapman and Hall Ltd.

Kimber DC and Gray CE., 1956 Textbook of Anatomy and Physiology. New York: The Macmillan Company.

Korolkovas A. 1970. essential of Molecular Pharmacology. Background for Drug Design. New York: Wiley-Interscience.

## **Bushmeat Hunting in North Sulawesi and Related Conservation Strategies (A case study at the Tangkoko Nature Reserve)**

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### **Abstracts**

*Over-hunting in North Sulawesi represents a serious threat to the local wildlife. Additionally, the illegal bushmeat trade due to the commercialized sale of wildlife for human consumption also threatens the future of many wildlife species in the region. The cultural and traditional practices of the local people have contributed to the over-hunting in this area. The market demand for the bushmeat is also threatening the long-term survival of many species. Local people in North Sulawesi have traditionally hunted wildlife, once abundant in protected sites such as the Tangkoko-Duasudara Nature Reserve and several other parts of Minahasa. A survey was conducted at three sites surrounding the reserve. The results showed that the species most frequently hunted were: macaca (11%), rat (38%), bat (22%), Sulawesi wild pig (17%), kuskus (6%) and birds (6%). The traditional hunting practices most commonly used included snare traps (45%), hunting dogs (35%) and shotguns (20%). If the level of bushmeat trade continues, the extinction of a number of North Sulawesi's endemic species is likely. Therefore, conservation efforts should be directed at an integrated management approach involving all components of the society including government, local community, and NGOs .*

**Keywords:** bushmeat, hunting, conservation

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## Introduction

Globally, hunting by local people has occurred for years. People mostly hunt wildlife for subsistence, as a source of protein. Wild meat provides more than 50 percent of the protein for many tropical forest people and is often a mainstay of their subsistence and cash economy (Alvard, 2002). Nowdays, hunting is one of the biggest threats to wildlife in worldwide. Forest protection alone is not sufficient since overhunting in many tropical countries is seriously depleting the natural populations of many forest animals. Loss of wildlife threatens the survival of the entire forest ecosystem. A decrease in crucial pollinators, seed dispersers etc. impacts species diversity and crubs the ability of a forest to maintain itself (i.e., reduced viability) after disturbance (Barret and Robinson, 2000). The impact of over-hunting affects people too. The sources of food, medicines and livelihood that indigenous communities depend upon are being severely depleted. Overexploitation is critically reducing vital human resources.

As in many other parts of the world, hunting pressure has become excessive in North Sulawesi. In this region, where the human population is predominately Christian, subsistent hunting and illegal bushmeat trade threatens the future of many wildlife species, including a number of endemic and endangered species (e.g., babirusa, anoa, Sulawesi black macaque). Clayton (2002) for example, has provided long-term datasets that describe the end market transactions by a wild pig dealer over a 10 year period. Additionally, the cultural and traditional practices of the local people also have triggered over-hunting in the region. Special occasions such as birthdays and christmas often include serving a meal of local wildlife. Moreover, local people believe that consuming wild meat may increase vitality and virility. As such, the market for wild meat remains in demand.

Local inhabitants not only hunt the protected animals, but they also hunt in the most protective areas, such as Tangkoko Nature Reserve located at the Northeast tip of the Minahasa peninsular. Populations of wildlife in this area have declined dramatically over the past few decades (Kyes et al., 2004). If this hunting practice continues, the extinction of a number endangerd species is likely. Already in Tangkoko, the babirusa and anoa have disappeared. Given the rate of hunting in North Sulawesi, closer monitoring is needed to define the scope of the overexploitation and provide data to assist with developing viable solutions/strategies to combat this problem.

This paper reports on the hunting practices of the local people living around the Tangkoko Nature Reserve. The main objectives were to identify the kinds of animals predominately hunted, describe the hunting methods used, and survey the local markets for the bushmeat species most commonly sold.

## Methods

The study was conducted from September-October 2005 in the three villages that border Tangkoko-Duasudara Nature Reserve (ie Pinangunian, Danowudu, and Dua Sudara). Tangkoko-Duasudara Nature Reserve is an 8867 ha nature reserve located at the northwestern tip of Sulawesi (figure 1).

From each village ten respondents known to be bushmeat hunters were selected for interview. Subjects were asked a set of questions to determine the kind of species they most frequently hunted and the hunting method used. In addition to questions specifically related to hunting and wild meat consumption, information concerning age and sex, religion, education level, income level, and profession was obtained.

Market surveys were carried out in Manado (Pasar Paldua, Pasar Kanaka) and Minahasa (Pasar Tomohon) to obtain information about the species that were sold in these traditional markets.

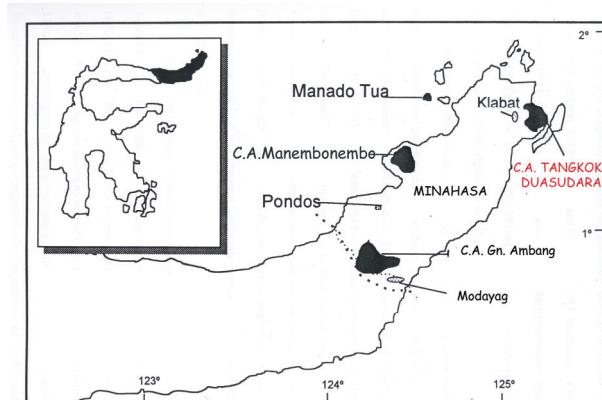


Figure 1. Study sites

## Results and Discussion

Survey of the three villages on Tangkoko Nature Reserve's border indicated that wildlife hunting activity was illegally done by the local people. Besides the demand to meet the daily supply for meat, some species such as the Sulawesi wild pig, rats, bats and macaca were captured since they were consider prized as pets (Dwiyah Herni et al 2001).

Species that were mostly frequently hunted were Macaca (18%), rat (38%), bat (22%), kuskus (6%) and birds (6%) (Figure 2.) Some of the meat was sold in the local market and the rest was consumed by the hunter and family. This is in line with the study of Lee. et.al 2004 that obtained that the largest portion of animal are hunted was rat which encountered which counted for 43% of rats, and followed by large bats (39.8%, small bats (7.5%) and sulawesi wild pig (7.3%) and cuscuses, anoa, babirusa, macaca are together counted for the remaining 1.2%

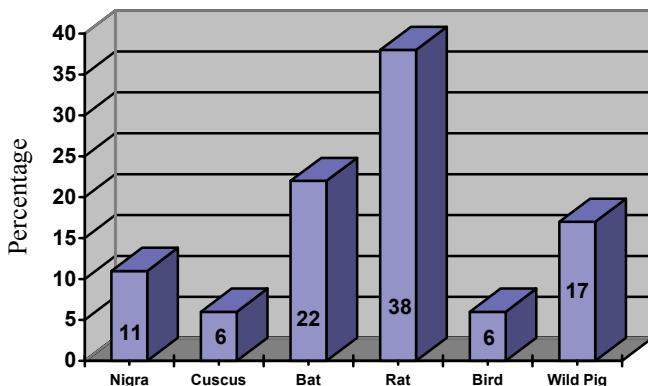


Figure 2. Species that are mostly hunted

The types of hunting methods used included snare trap, hunting dog, and shotgun (figure 3). These traditional hunting methods are not changed significantly over the years.

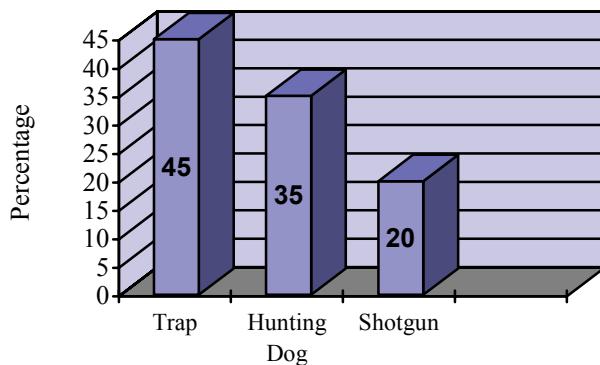


Figure 3. Hunting method

Surveys of the three local markets in Manado and Minahasa indicated that several wildlife species were sold as live animals or as a processed meat. Bushmeat was mostly sold on Saturdays and species mostly preferred by the local people were sulawesi wild pig, bats, rats and the Sulawesi black macaca (figure 4).



Figure 4. Animals sold on the local market

At present, many wildlife species are continually being hunted illegally. This is related in part to the traditional and cultural practices in North Sulawesi particularly in Minahasa where the local people eat bushmeat on a regular basis and on special occasions such as birthdays and Christmas. Moreover, the desire to eat bushmeat species that are difficult to find, such as macaca, is increasing since such species are viewed as more exotic. In north Sulawesi, the Minahasa people consider wild pig meat to be superior to domestic pork, and are willing to pay 20% to 50% more for it. Reports obtained during brief surveys of the three village markets in north-east Sulawesi, suggest that butchers in each market handled from 2 to 20 wild pigs per week, buying them live from commercial hunters and slaughtering them when needed.

The continuing hunting practice is mostly due to the lack of infrastructure and management of the protected areas of North Sulawesi. In addition to this, other threats such as forest fires and forest conversion for agricultural purpose contribute to further to the decline of the wildlife populations.

To overcome these problems some realistic, long-term conservation strategies are needed. Further, the successful conservation program will require an integrated approach involving the participation of all components of the society (i.e. government, local community, and NGOs). Practical efforts to combat the illegal bushmeat hunting include establishing an effective monitoring program in protected areas, controlling the bushmeat trade, establishing a practical system of law enforcement to control illegal hunting, and promoting education and awareness programs on conservation.

### **Conclusion**

Hunting and the commercial sale of bushmeat in North Sulawesi is continuing at a high level. If this level of bushmeat trade continues, the extinction of a number of NS's endemic species is likely. Therefore, conservation efforts should be directed at an integrated management approach involving all components of the society.

### **References**

Macdonald. AA. 1993. The Sulawesi Warty Pig (*Sus celebensis*) Status and Action Plan Summary

Alvard, M.S., 2000. The impact of traditional subsistence hunting and trapping on prey populations: data from Wana horticulturalists of upland Central Sulawesi, Indonesia. In: Robinson, J.G., Bennett, eds. Hunting for Sustainability in Tropical Forests.

Bennett, E.L. and J.G. Robinson. 2000. Hunting of Wildlife in Tropical Forest. The World Bank Environtment Department.

Clayton, I.M. and E.J. Milner - Gulland. 2002. The Trade of Wildlife in North Sulawesi, Indonesia. In: J.G.Robinson and E.L. Bennett eds. Hunting for Sustainability in Tropical Forests.

Kyes, R.C., Perwitisari, D., Iskandar, E., Onibala, J., Jones - Engel, L., Paputungan, U., Nangoy, M., Ferguson, B., Grant, R., and Pamungkas, J. 2004. Assessing the abundance and genetic status of the *Macaca nigra* at Tangkoko Nature Reserve, Indonesia. *Folia Primatologic*, 75(1), 388.

Lee, R. J., A. J. Gorog, A. Dwiyahreni, S. Siwu, J. Riley, H. Alexander, G. D. Paoli, and W. Ramono. 2005. Wildlife trade and implications for law enforcement in Indonesia: A case study from North Sulawesi. *Biological Conservation* 123:477-488.

# **Establishment of Sustainable Signal Grass Pasture by Amendment of *Chromolaena odorata* Biomass and Manure as Nutrient Organic Source: Effect on growth parameters, dry matter production and carrying capacity.**

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## ***Abstract***

*In many tropical pastures invasive weed like Chromolaena odorata becomes a serious species with no redeeming feature and causes poor and low calving rates of local cattle. Utilization of C. odorata biomass as organic nutrient source may be an alternative management to eliminate the distribution of the weed and improve pasture productivity.*

*A field study in mini pastures was conducted to recognize annual forage production (AFP), carrying capacity, N- and P Uptake, and protein production of signal grass grown on soil amended with C. odorata biomass and feces as organic nutrient source. Block Randomized Design consisting of: no treatment (blank control = P0); 7.2 kg plot<sup>1</sup> of C. odorata (PC); 21 kg plot<sup>1</sup> of manure (PF); combination of C. odorata (3.6 kg plot<sup>1</sup>) and manure (10,5 kg plot<sup>1</sup>) (PCF) and inorganic fertilizer (573.3 g urea plot<sup>1</sup> and 217 g super phosphate plot<sup>1</sup> (positive control=PA), with 4 replications. Carrying capacity was calculated according to simulation of accumulate grass production throughout the year. Dried herbage was use to determine forage production, N-and P uptake. Protein production was calculated from N concentration, 6.25 factor and forage production.*

*The results showed that PC improved ( $p<0.01$ ) AFP about 225% and 110% as compared to P0 and PF, respectively. PC and PF are able to substitute inorganic fertilizer about 60% and 50%, respectively in resulting similar AFP as compared with those of PA. PC and both PF and PCF increased ( $p<0.05$ ) carrying capacity of the pasture up to 1.7 and 1.3 Animal Unit, respectively as compared with P0. PC, PF and PCF produced higher protein production ( $p<0.05$ ) than P0, and substituted to inorganic fertilizer by 46%, 40% and 49%, respectively.*

**Keywords:** *Brachiaria humidicola, Chromolaena odorata, manure,*

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## Introduction

### Background

High intensity of pasture use due to intensive animal rearing for replacement stock leads to soil and nutrient degradation. In many pasture area in Indonesia, most of degraded pasturelands are invaded by invasive weed species like *Chromolaena odorata*. It is a perennial species and has other name *Eupatorium odoratum* L., *E. Conyzoides* Vahl and *Osmia odorata* (L.) Schultz - Bip (Hanum dan Maesen, 1997). In Indonesia it is known with name Kirinyu or Babanjaran (Tjitrosoedirdjo *et al.*, 2002 dan Sipayung *et al.*, 2002). It becomes a serious species with no redeeming feature and causes poor and low calving rates of local cattle. This is because *Chromolaena* grows very aggressive and has ability to intensive sprouting. Those enable changing of botanical composition and reduction of pasture quality and causing toxic to animals. Our previous study records that reduction of pasture area due to *Chromolaena* invasion ranged 8-15% a year depending on grazing intensity.

To maintain pastureland and eradicate *Chromolaena*, an alternative management has been studied in this experiment to utilize its biomass as mulch material, rather than eradicating with chemical agent because it is harmful to animals. From chemical composition view point of *Chromolaena* indicated high quality of mulch material, because rate of decomposition and nutrient mineralization is affected by both nutrient content and chemical composition of the plant material (Abdullah, 2002; Bossuyt *et al.* (2001); Breland (1997). *Chromolaena* has a relatively high quality as compared to other weed species. It's leaves have a lower C/N ratio (25.8%) and C/P ratio (395), lower lignin (13.1%), ADF (53.3%) and cellulose (40.2%) content than common pastoral weed species (Abdullah, 2002).

Base on its chemical composition, it is expected that amendment of *Chromolaena* as mulch material (organic nutrient) can supply nutrient into soil slowly, through decomposition process and mineralization. The presence of fungi and bacteria in soil cause the major chemical transformations in decomposition, e.g. the degradation of polysaccharide complexes of plant litter to carbon dioxide, and mineralization of protein to ammonium and nitrogen and organic phosphorus to inorganic P. The soil microorganism activities in soil during decomposition are strongly influenced by soil moisture content (Taylor *et al.*, 1999), litter type and fertilizer (Donnison *et al.*, 2000), and temperature (Grisi *et al.*, 1998)

## **Objectives**

The objective of the experiment were to recognize the effect of *Chromolaena* biomass compared with conventional fertilizer (manure and inorganic fertilizer) on signal grass growth, dry matter production and calculated carrying capacity, and to investigate the contribution of *Chromolaena* amendment able to compensate inorganic fertilizer.

## **Materials and Methods**

### **Time and Location**

The field experiment was conducted in 2004 in research station of Grassland science division Faculty of Animal Scince, Bogor Agricultural University (IPB). The site has two seasons, the rainy season with seven to eight consecutive wet months and the dry season up to four consecutive dry months. The average monthly rainfall during experiment was  $364 \text{ mm month}^{-1}$  ( $4375 \text{ mm year}^{-1}$ ). The highest rainfall intensity was on April with 639 mm (25 days) and the lowest rainfall intensity was on June (169 mm, 8 days), indicating that at the beginning of June the dry season started.

### **Signal grass plots**

Experimental plots were already prepared one year before experiment. There was no agronomical treatment before the plots used in the experiment. Signal grass had well established and produced 594.9 g/plot/harvest or 2092 g/plot/year (3487 kg/ha/year). The plots were made by marking each border of plots, and separated with bare area (50 cm). Before application of fertilizer, signal grass was trimmed to avoid physiological effect of grass individual.

### **Mulch material and manure preparation**

*Chromolaena* biomass (before flowering stage) was collected from pasture area that invaded grazing area of university grassland. The collected biomass was chopped with 10cm length before application. The amount of biomass as mulch material (consisting of all above ground biomass) applied on each plot was 12 ton fresh weight/ha (3 folds of potential biomass production of *Chromolaena*). This amount of applied biomass contributed to 398 kg N/ha and 186 kg P/ha. According to laboratory analysis, nitrogen and phosphorus content of

*Chromolaena* biomass were 3.32% and 0.16%, respectively. Base on those calculations, the amount of biomass applied on each plot was 7.2 kg fresh weight/plot

Nitrogen and phosphorus content of manure were analyzed before application. The N and P content of the manure were 1.13% and 0.37%. Manure was collected from university farm. The manure originated from manure produced by 1.5 animal units (this animal numbers was accordingly the carrying capacity of signal grass pasture each ha/year). The amount of applied manure was 4 folds of manure production (54,750 kg fresh weight/ha or 21 kg fresh weight/ha). This amount of manure contributed to about 618 kg N/ha and 130 kg P/ha. Based on this calculation, for combination treatment, the amount of both *Chromolaena* and manure were a half of single treatment dosage (3.6 kg/plot and *Chromolaena* 10.5 kg/plot).

The Inorganic fertilizers used in the experiment were urea (45% N) and SP-36 (36% P<sub>2</sub>O<sub>5</sub>). The dosage of applied urea and SP-36 was based on N and P supply contributed from both *Chromolaena* and manure i.e.: 895.6 kg urea/ha and 361.1 kg SP-36/ha (537.3 g/plot and 217 g/plot, respectively).

### ***Mulch and Fertilizer application***

Mulch and manure were applied (5 cm thick) directly on the top of trimmed signal grass according to experimental design. Urea and SP-36 were applied using broadcast method on trimmed grass surface. A half dosage was applied at the beginning of experiment and other half dosage was applied prior to dry season.

### ***Parameters observations***

During growing, signal grass was taken care. Some parameters including number- and length of stolon (primer and secondary stolon), tiller number and tillering rate were measured every two weeks. To enable measuring stolon length and counting tiller numbers at certain grass individual, observed individual of grasses were marked by using different color of pennant. Primer stolons were main stolon that grew directly from main crown and produced tillers (daughter tiller) at their nodes. Secondary stolons were branches of stolon that grew from daughter tillers and produced grand daughter tillers from their nodes (see Fig. 1). Tiller numbers consisting of daughter and grand daughter tillers were calculated from plots every two weeks. Tillering rate was

ratio of grand daughter tiller number to daughter tiller number resulted within a week.

Dry matter production was investigated every harvesting time. Sample of dry forage was analyzed to recognize the N and P content of the forage. Forage was harvest every 60 days, and dry matter production was accumulated according to seasons (dry season and rainy season).

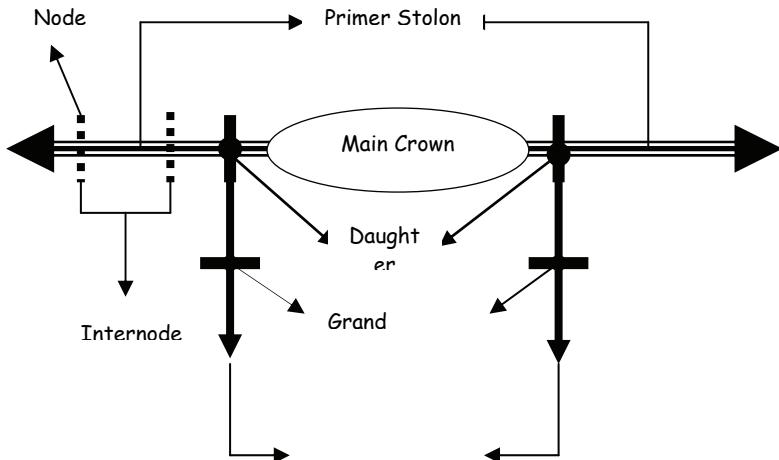


Fig. 1. Scheme of tillers and stolons

### **Experimental design**

The fertilizer sources, which were used as treatments, originated from *Chromolaena* biomass, manure (cow manure) and inorganic N and P fertilizer (Urea and SP-36). The experimental design used in this study was block randomized design, consisting of : PO = blank control, PC = *Chromolaena* biomass (7,2 kg fresh weight/plot), PF = manure (21 kg fresh weight/plot), PC+F = combination of *Chromolaena* biomass (3,6 kg fresh weight/plot) and manure (10,5 kg fresh weight/plot), Pi = inorganic fertilizer (urea 537,3 g/plot and SP-36 217 g/plot) as an positive control. Each treatment was repeated 4 replicates, so that the number of experimental plots was 20 plots. Collected data were analyzed using analyses of variance, and significant differences of average data on each treatment were tested with orthogonal contrast

(Steel and Torrie, 1991), and compared with blank control and positive control.

## Results and Discussions

### Effect of Fertilizer on Growth parameters

Application of *Chromolaena* as mulch material and manure as single treatment and their combinations significantly increased primer stolon number ( $P<0,01$ ), secondary stolon number, daughter tiller and length of stolon, length rate of primer stolon ( $p<0.05$ ) as compared with blank control (Tabel 1.). However fertilizer application did not significantly affect grand daughter tiller number, tillering rate and length of secondary stolon. Application of either *Chromolaena* or manure as single treatment and their combination significantly doubled primer stolon comparable to blank control. However, Combination of both *Chromolaena* and manure resulted in lower stolon number than those of plot applied with *Chromolaena* or manure as single nutrient source. Different from primer stolon, application of *Chromolaena* and manure did not affect secondary stolon number, but application of inorganic fertilizer resulted in highest stolon number.

Tabel 1. Effect of different nutrient sources on growth parameters of signal grass

Parameters	Fertilizer application				
	PO	PC	PF	PC+F	Pi
Primer stolon number (stolon/plot)	2,3 <sup>d</sup>	5,5 <sup>b</sup>	5,2 <sup>b</sup>	4,9 <sup>c</sup>	13,7 <sup>a</sup>
Secondary stolon number (stolon/plot)	0 <sup>b</sup>	1 <sup>b</sup>	0,8 <sup>b</sup>	0,3 <sup>b</sup>	3 <sup>a</sup>
Daughter tiller number (tillers/pot)	0,75 <sup>b</sup>	8,62 <sup>a</sup>	9,28 <sup>a</sup>	8,97 <sup>a</sup>	7,59 <sup>a</sup>
Grand daughter tiller no. (tillers/plot)	0	1.75	0.77	0.25	0.74
Tillering Rate (no./week)	0	0.18	0.07	0.02	0.22
Length of stolon (cm/week)	5,8 <sup>c</sup>	20,3 <sup>a</sup>	17,2 <sup>a</sup>	14,7 <sup>b</sup>	20,3 <sup>a</sup>
Length rate of primer stolon (cm/week)	0 <sup>c</sup>	7,5 <sup>a</sup>	3,2 <sup>b</sup>	3,3 <sup>b</sup>	6,9 <sup>a</sup>
Length rate of secondary stolon (cm/week)	2,07	2,53	2,61	2,71	2,70

PO = blank control, PC = *Chromolaena* amendment, PF = manure application, PC+F = combination of *Chromolaena* and manure, Pi = inorganic fertilizer

Application of *Chromolaena* or manure, and their combination resulted in significant higher tiller number of daughter (8-9 folds) and

length of stolon as compared with control, but has no different tiller number of daughter as compared with inorganic fertilizer (Tabel 1). However combination of *Chromolaena* and manure resulted in lower length of stolon than those of other three fertilizer application. *Chromolaena* and manure application was able to substitute 35%-39% the use of inorganic fertilizer to result in a same number of grass stolon.

### **Forage production**

Grass production drastically increased ( $p<0.01$ ) if the plot was applied with fertilization. As depicted in Table 2, amendment of *Chromolaena*, manure and their combination as nutrient sources for the grass improved respectively about 139% and 100% of average DM production during rainy season. Application of inorganic fertilizer resulted in the highest average DM production. It is shown in Table 2, that *Chromolaena* application resulted in higher DM production than those of either manure or combined fertilizer.

Table 1. Effect of different nutrient sources on dry matter production of signal grass and ruminant carrying capacity

Parameters	Fertilizer application				
	PO	PC	PF	PC+F	Pi
Average DM production in rainy season (g/plot)	512 <sup>d</sup>	1224 <sup>b</sup>	1024 <sup>c</sup>	1046 <sup>c</sup>	203 <sup>4a</sup>
Average DM production in dry season (g/plot)	282 <sup>d</sup>	422 <sup>c</sup>	495 <sup>b</sup>	443 <sup>c</sup>	738 <sup>a</sup>
Cumulative DM production (kg/ha/year)*	3043 <sup>d</sup>	6857 <sup>b</sup>	5973 <sup>c</sup>	5997 <sup>c</sup>	114 <sup>57a</sup>
Calculated animal carrying capacity (ST/ha/year)*	1.3 <sup>c</sup>	3.0 <sup>b</sup>	2.6 <sup>b</sup>	2.6 <sup>b</sup>	5.0 <sup>a</sup>

PO = blank control, PC = *Chromolaena* amendment, PF = manure application, PC+F = combination of *Chromolaena* and manure, Pi = inorganic fertilizer

During dry season, grass production reduced drastically, in particular grasses that grew on the plots fertilized with *Chromolaena* biomass and inorganic fertilizer. It may be because *Chromolaena* and inorganic fertilizer can supply faster inorganic nutrient than manure and combined fertilizer. This was approved by research results of our previous study finding that *Chromolaena* was the fastest degradable materials and very fast recovery of mineralization, that indicate fast release and fast supply of organic minerals.

Application of *Chromolaena* resulted in higher DM production ( $p<0.05$ ), as well as combination of *Chromolaena* and manure. Application of manure as single fertilizer led to produce higher grass

production than those of *Chromolaena* or combined nutrient sources. Application of inorganic nutrient resulted in the highest grass production.

Cumulative grass production was significantly influenced ( $p<0.01$ ) by *Chromolaena* amendment, manure application and inorganic fertilizer. Application of *Chromolaena* doubled cumulative grass production, and manure and combined fertilizer application as well. Application of inorganic fertilizer resulted in the highest cumulative DM production of signal grass. Application of organic nutrient sources can only reach a half of grass production comparable to inorganic nutrient sources.

Carrying capacity is the capability of area that can supply forage for animals throughout a year without causing destruction of the pasture area. The carrying capacity was calculated and based on cumulative dry matter production of the grass and converted to one ha. It is assumed that animal consumes 6.29 kg DM of grass/day/head (Indonesian condition). Application of organic nutrient sources originating from *Chromolaena*, manure and their combination significantly increased 1.3-1.7 animal units. Application of inorganic fertilizer increased carrying capacity about 3.7 animal units.

Amendment of *Chromolaena* biomass into soil improves nutrient supply 32 days after application (Abdullah, 2002). Our results confirmed this finding, and showed that amendment of *Chromolaena* biomass at the beginning of application led to immobilization of released nutrient by soil microorganisms. This was indicated by yellow leaves of signal grass at the beginning of application, but then the grass grew better and leaves showed green.

Increased tiller number and dry matter production was associated with increase of stolon number and stolon length. Application of fertilizer gave more chance to grass to extent their stolones. There was a tendency that application of combine organic fertilizers (*Chromolaena* and manure) led to slower growth than application of them as single treatment. In general, it can be mentioned that application of *Chromolaena* as mulch material resulted in better growth performance of signal grass.

## Conclusions

Amendment of biomass originated from *Chromolaena* and manure and their combination improved signal grass growth, production

and carrying capacity and could substitute about 50% of chemical fertilizer (urea and SP-36). Use of *Chromolaena* may be an alternative pasture management to sustain quality and production of signal grass pasture.

## References

Abdullah, L. 2001. P-mineralization and immobilization as result of use of follow vegetation biomass in slash and mulch system. Disertasi. Cuviller Verlag, Gottigen.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R. and Paustian, K. 2001. Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology*, Vol. 16 (3): 195-208.

Breland, T.A. 1997. Modeling mineralization of plant residues: Effect of physical protection. *Biol. Fertil. Soils*, 25: 233-239.

Donnison, L.M., Griffith, G.S. and Bardgett, R.D. 2000 Determinants of fungal growth and activity in botanically diverse hay meadow: effects of litter type and fertilizer addition. *Soil Biology and Biochemistry*, 32(2000) 289-294

Grisi, B., Grace, C., Brooks, P.C., Benedetti, A. and Dell'abate, M.T. 1998. Temperature effects on organic matter and microbial biomass dynamics in temperate and tropical soils. *Soil Biology and Biochemistry*, 30(10-11): 1309-1315.

Hanum, F. dan L. J. G. van der Maesen. 1997. Plant resources of South-East Asia. Prosea. Bogor, Indonesia.

Sipayung, A., R. D. Chenon and Sudharto Ps. 2002. Observation on *Chromolaena odorata* (L.) R. M. King and H. Robinson in Indonesia. *Contens: Second International Workshop*. <http://www.cpitt.uq.edu.au/chromolaena/2/sipay.html> [30 Juli 2002]

Steel, R. G. D., dan J. H. Torrie. 1991. Prinsip dan Prosedur Statistik. Suatu Pendekatan Biometrik. Terjemahan. M. Syah. PT Gramedia, Jakarta.

Taylor, L.A., Arthur, M.A. and Yanai, R.M. 1999. Forest floor microbial biomass across a northern hardwood successional sequence. *Soil Biology and Biochemistry*, 31(3):431-439.

Tjitosoedirdjo, S., S. S. Tjitosoedirdjo and R. C. Umaly. 2002. The status of *Chromolaena odorata* (L.) R. M. King and H. Robinson in Indonesia. *Contens: Second International Workshop*. <http://www.cpitt.uq.edu.au/chromolena/2/umaly.html> [30 Juli 2002]

# **Impacts of Pigs Farming on the Living Environment at “Pakakaan Zone”**

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## **Introduction**

Animal husbandry is important for human being as animal protein source for their life. Therefore, farming area development need to be developed and preserve sustainably. Pig farming represents one of livestock source for very efficient accomplishment of nutrition, so that the economic meaning of livestock volume will be higher. In other words pig livestock use for animals protein accomplishment can also improved regional and national earnings.

Pig livestock in Indonesia developed in certain areas like in Minahasa area; called Pakakaan zone taken from four sub-districts or villages of (TomPAso, KAKas, KAwangkoan and langowan) whereas, the majority resident of non Moslem believers.

Pakakaan is an agropolitan zone aim as the field to develop agribusiness system integrated by Oriental Market Strategy and by developed the infrastructures such as guilty house, market, feeding producers and road development.

Kawangkoan sub-district addresses to be a centre of pig livestock. The populations in Kawangkoan sub-district are 3.138 heads, East Langowan sub-district are 2.660 heads, whereas in East Langowan sub-district have the biggest market among Pakakaan and in Minahasa. The type of pigs farmed source from the type of Landrace and Yorkshire.

The pig farming require to be studies especially the impact weather negatively or positively, the meaning of the impact is an environment change in the base of farming activities. By studying the impact of pig farming to the stakeholder environment can be an idea to build the Pakakaan areas continually in environment area based on the indigenous knowledge of the people of Pakakaan.

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## Materials and Methods

### ***Data Collecting.***

This research done by using the merger between PRA (Participative Rural Appraisal methods and PLA (Participative Local Appraisal) in Kiawa II Kecamatan Kawangkoan and Sumaraya village of Kecamatan East Langowan. The two villages chosen have criteria as follows:

1. Have the most of pig farming.
2. The pig farming is around the resident settlement.
3. Emited the stream of Ranowangko river (Kiawa II village, Tondano sub stream and Sumaraya village.

This research held for a month from 1<sup>st</sup> September to 30<sup>th</sup> September 2006.

The technique to collect data as follows:

1. Structuring interview for seventy respondents (include the key respondents)
2. Focused group discussion, for filter information from farmers' group.
3. Visiting farmers and villages around pig farming.
4. Temporal diagramming to collect the developing data of pig farming.
5. Transect walk conduct to equip field data. This activity used to perceive farm activities include horses and cows ranches.
6. Secondary data collections:
  1. From relevant institutions (include village and sub-district monograph).
  2. Reverences include result research like script, thesis and A2 program research of social economy Department of Faculty of Animal Husbandry Sam Ratulangi University.

### **Data Analysis**

Analysis is used system analysis by geographical model (Causal loop =

In the form of diagram emit a stream of or flow chart), and verbally by questionnaires, and mathematically to calculate the earnings of farmers, and the relation of impact generated with perception.

## Result & Discussion

### *The general fact of Pig Livestock.*

The perception to the pig farm in Pakakaan field was not match to the legal and regulation used in our regional, among others the pig farming lied in the settlement area, in other words they disagree according to the decree of Director General of Livestock Services No. 7756/KPTS/DJP/Deptan/1982 about technical conditions of General Farming on Pig Company.

Chapter 1 Section 1 Point b. and c., they are:

1. It cannot lay in resident settlement in downtown with the 1000 meters distance.
2. It has to pay attention to environmental, in such a manner so that waste mutilation, pickings and cannot contaminate region outside the farm.

### *The effect of Pig farming to the environmental.*

1. The activity of cage sweeping yield waste produce (volume of waste 1,6m<sup>3</sup>/tail/year, BOD5 with 28,4 Kg/tail/year, SS 183 kg/tail/year and N total 8,4 kg/tail/year.) They can produce air quality change, as well as land quality change and water quality change. The result of perception directly in these two villages showed that the air quality became lower by the production of process of obsolesce by reek resulted and more flies especially in the evening. From 70 respondents, 60 respondents evade this existence, but more and more they become accustomed to the obsolescence, they accept this problem, but sometimes they cannot bear and became headache. For land quality and the water quality index became lower. The result of the research showed changing of land and water quality making the corn plants produce abnormally. But these impacts did not affect the farmers' in Sumarayar village, from the questionnaires answers showed 50% of respondents produce corn plants altogether with Karombasan grass of letup grass or hard grass for horses and cows. These grasses support the development of cows and horses ranches. The populations of horses and cows in these two district (kecamatan) bigger than Kawangkoan District, especially cows (1,809 heads), to horses (202 heads), horses (680 heads). Economically, the corn plants as their grasses give more profit than just concentrate as feed, produce 2,5 months have Rp. 500,- space for a cluster of corn green leafs converted to 1 Kg of concentrate (one cluster of corn green leafs is Rp. 3500,- and the value of Rp. 3000 for a kg concentrate). On

other side the lowering of Water Quality Index resulting the raising of BOD5, SS and N total. Raised the blooming Eceng Gondok (*Echornia crassipes*) in Tondano lake and those plants bother the activities of PLTA Tanggari (“until these days there is no research especially about the impact of pig farming to *Echornia crassipes* plants blooming in Tondano lake and the assume of sedimentation volume in Watershed basin of Tondano and also the volume of big waste resulting”). The different of water analysis in upriver irrigation and in estuary of Watershed basin of Tondano as follows:

Up river: TDS 228,7; Estuary 14,27 mg/l.

The change showed the economy activities include pig farming in drainage basin of Tondano, beside that those place showed biodiversity change of watershed basin. From the result of farmers fish interviewing in lake Tondano, Eceng Gondok (*Echornia crassipes*) exploited used for irrigation purification, to prevent moss production bother them as fish farmers in floating net (Massie, 2000)

- Martketing and Labour Recruitment Activity.

The financial analysis result of pig farming (Makalew et al.,2006). The profit per tail Rp. 413.716 and the average of R/C value is 1,94. (1,45 – 2,38) but financially pig farming is efficient. The raising of the earnings relate to the education, from the interview result generally to get study more because of pig farming. Two respondents give more study to their son to go to medicine faculty in university of Sam Ratulangi.

- Flesh Consumption.

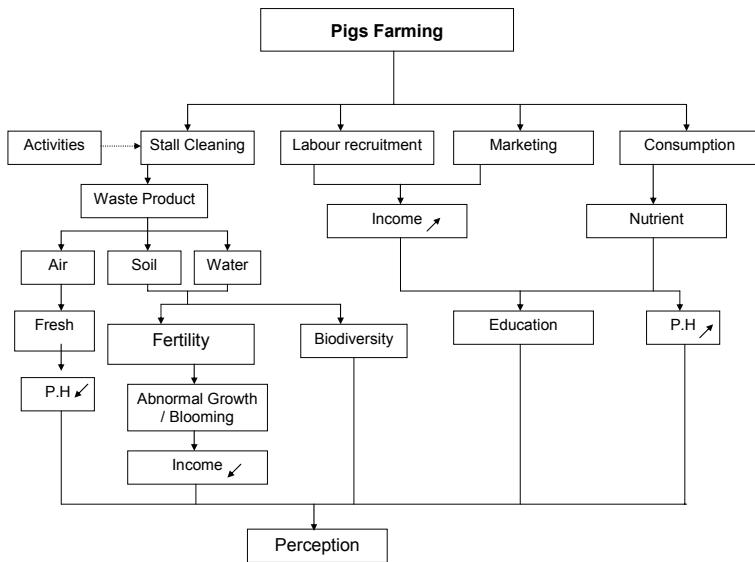
From the research result of people (100 % respondents) consume pig flesh (1 kg/week/family). It is a habit of people in this area on arranging a special food in Sunday, and also on special day like Christmas, New Year, and Easter day, they used to buy together pigs still going on until today. For instance 70-90 kgs of pig bought by four families. In other words for fulfill the national standard was done.

## 2. Impacts of the Region.

The result of analysis by using Spearman Equation model indicating that:

The perception relation by the earnings RS = 73,79, the perception with the oldly 73,79 > 1,67 by (table t 0,05).

In other words, the people of pig farming say that growing pigs is an alternative way to give earnings to farmers positively. The analysis result to the surrounding people, have the perception with education level = RS 75,39 of perception with age level 19,64. To be more clearly the impact of pig farming in Pakakaan zone showed the picture of sigma by “flow chart” of the impact of pig farming to the environment of Pakakaan zone.



## Conclusion

The pig farming give impact in Pakakaan zone, both for having positive and negative parameters of air, land and water, as well as the parameters of economy perception, and the health of people perception.

## Suggestion

1. It is required to perform a continue research of livestock of pig farming to thyroid blooming in Tondano lake and superficiality with analyzing quality of water, land and ground.

2. Require to be tested the air quality and pig farming as livestock by laboratory work.

## References

Canter, L.W. (1977). Environment Impact Assesment Mc. Graw and Hill Book Company, New York.

Krider, J.L, W.E Carroll. 1971. Swine Production. Fourth edition. TATA McGRAW-HILL PUBL. COMP. LTD. Bombay – New Delhi.

Makalew, A., M.T. Massie, R.A.J. Apituley, J.C. Loing, J.A. Malingkas. 2006. Analisis Finansial Usaha Ternak Babi di Kecamatan Kawangkoan Kawasan Agropolitan PAKAKAAN. Program A2 Jurusan Sosial Ekonomi FAPET UNSRAT MANADO.

Massie, M.T. 2000. Identifikasi kegiatan-kegiatan perekonomian masyarakat di Hulu-Tengah DAS Tondano. Karya Ilmiah Penelitian. PPLH – SDA Unsrat Manado.

Mitchell, B., B. Setiawan., D.H. Rahmi. 2000. Pengelolaan Sumberdaya dan Lingkungan. Gajah Mada Univ. Press. Yogyakarta.

Muhammad, F., H. Jaali. 2003. Metodelogi Penelitian Sosial (Bunga Rampai). PTIK Press bekerja sama dengan CV Restu Agung. Jakarta.

Peraturan Pemerintah Republik Indonesia No. 27 Tahun 1999 tentang Analisis Dampak Lingkungan Hidup. BAPEDAL Regional III.

Purba,J. 2005. Pengelolaan Lingkungan Sosial. Yayasan Obor Indonesia Jakarta.

Surat Keputusan DIRJEN Peternakan No. 775/KPTS/DJP/DEPTAN/1992 tentang Syarat-syarat Tehnis Perusahaan Peternakan Babi.

Soerjani, M. , A, Yuwono. , D, Fardiaz. 2005. Lingkungan Hidup (The Living Environment ). Yayasan Institut Pendidikan dan Pengembangan Lingkungan. Jakarta.

# Improving Quality of Local Feedstuff and Its Use for Fattening Of Peranakan Ongole (PO) Male Cattle

Muhamad Bata<sup>1)</sup>

## Abstract

*Rice straw is by-product have not fully been used to feed ruminant especially cattle because of low quality indicated by low nitrogen content and fermentable carbohydrates, while ligno cellulolytic and hemicellulolytic bound are high. Those limitations will cause negative effect of rumen microorganism development and the cattle when they are fed it. Improving quality of rice straw should be done and one alternative of the treatments to solve these problems is ammoniation by using urea. Those treatments increase nitrogen content and its degradability in the rumen and also breakdown ligno cellulolytic and hemicellulolytic, while fermentable carbohydrate as energy for rumen microbes is limited. Therefore, the utilization of rice straw ammoniated should be supplemented with other feedstuff that high fermentable carbohydrates. Fresh cassava waste is by-product from cassava processing that still has remained of starch. It can be used as fermentable energy source for cattle fed rice straw ammoniated; however, the limitation of that feedstuff is high crude fibre. The objective of this research was to improve quality of fresh cassava waste by treating with fibrolytic enzymes as fermentable energy source on performances fattening of local male cattle fed rice straw ammoniated. Sixteen 18 mouth old male local cattle of Peranakan Ongole (PO) were divided into 4 groups based on initial body weight as block. The average of body weight of each group was I= 250,5 kg, II= 218 kg, III= 204,5 kg, IV= 186,4 kg, therefore, Completely Randomised Block Design (CRBD) was used for this experiment. As treatments were kind of diets i.e. A, B, C and D. This diet A diet consisting of fresh cassava waste, rice bran, soybean cake waste, copra meal, mineral mix and salt as concentrates with 14 CP. This diet was used as control (diet A). In treatment diet B, C and D were adding of fibrolytic enzyme to fresh cassava waste 12 hour before mixing to other feedstuff of concentrates for 0,75, 1,5 and 2,25 g/kg dry matter (DM), respectively. The animals were fed with rice straw ammoniated and concentrate at 3% of body weight (BW) on DM basis. The concentrate to rice straw ammoniated DM ratio was maintained at 60: 40, respectively. Variables measured were digestibility of dry matter (DM) and organic matter (OM), average daily gain (ADG), DM intake (DMI), Feed efficiency (FE) and Feed Conversion (FC). The result showed that adding fibrolytic enzyme to cassava waste was not significant effect on ADG, DMI, FE and FC. However, cattle received B, C and D diet tended to have lower ADG and FE compared to the control. The average of ADG and FE were 1,07 kg, 1,00 kg, 1,02 kg, 1,01 and 13,71, 12,68, 13,15, 13,61 for A, B, C and D, respectively. It can be concluded that fresh cassava waste can be used as fermentable energy when cattle fed rice straw ammoniated without any treatments.*

**Key word:** fibrolytic, enzyme, cattle, rice straw, cassava waste.

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## Introduction

Microbial yield in the rumen depends largely on the availability of carbohydrates and N in the rumen. Synchronization of the rate of carbohydrate fermentation and protein degradation has been suggested as a means to optimize microbial growth in the rumen (Hoover and Stokes. 1991). Although in some in vitro studies (Ørskov and McDonald. 1979), bacterial yield was increased by a synchronous supply of N and carbohydrate, in other studies (11), no improvement was observed. Studies with lactating cows showed alterations in microbial fermentation in the rumen that changed the concentration of ruminal metabolites (Stokes et al. 1991 and Arieli, 1996) or affected milk yield and composition (Aharoni et al., 1993 and Aldrich et al., 1993) when the dietary concentrations of ruminally degradable carbohydrate and protein were varied. Robinson and McQueen (1994) found no effect on ruminal VFA concentration or overall milk yield from the synchronization of ruminal fermentation of carbohydrate and N in the diet. Diets synchronized on a daily basis using the true ruminal degradability of protein and carbohydrate may have an imbalance between the fermentable carbohydrate and N supplies on an hourly basis.

Rice straw is by-product have not fully been used to feed ruminant especially cattle because of low quality indicated by low nitrogen content and fermentable carbohydrates, and also is high of ligno cellulolytic and hemicellulolytic bound. Those limitations will cause negative effect of rumen microorganism development and the cattle when they are fed it. Improving quality of rice straw should be done and one alternative of the treatments to solve these problems is ammoniating by using urea. Those treatments increase nitrogen content and its degradability in the rumen and also breakdown ligno cellulolytic and hemicellulolytic, while fermentable carbohydrates as energy for rumen microbes are limited. Therefore, the utilization of rice straw ammoniated should be supplemented with other feedstuff that high fermentable carbohydrates. Fresh cassava waste is by-product from cassava processing that still has remained of starch. It can be used as fermentable energy source for cattle fed rice straw ammoniated; however, the limitation of that feedstuff is high crude fibre. The objective of this research was to improve quality of fresh cassava waste by treating with fibrolytic enzymes as fermentable energy source on performances fattening of local male cattle fed rice straw ammoniated.

## Materials and Methods

Sixteen 18 mouth old male local cattle of Peranakan Ongole (PO) were divided into 4 groups based on the initial body weight (BW) as block. The average of body weight of each group was I= 250,5 kg, II= 218 kg, III= 204,5 kg, IV= 186,4 kg, therefore, Completely Randomised Block Design (CRBD) was used for this experiment. As treatments were kind of diets i.e. A, B, C and D. This diet a diet consisting of fresh cassava waste, rice bran, soybean cake waste, copra meal, mineral mix and salt as concentrates with 14 CP. This diet was used as control (diet A). In treatment diet B, C and D were adding of fibrolytic enzyme to fresh cassava waste for 0,75, 1,5 and 2,25 g/kg dry matter (DM), respectively. The animals were fed with rice straw ammoniated and concentrate at 3% of body weight (BW) on DM basis. The concentrate to rice straw ammoniated DM ratio was maintained at 60: 40, respectively. The composition and nutrient content of experiment diets were presented at Table 1. Variables measured were digestibility of dry matter (DM) and organic matter (OM) by using total collection method (Krause et al., 1998), average daily gain (ADG), DM intake (DMI), Feed efficiency (FE) and Feed Conversion (FC).

Table 1. The composition and nutrient content of feedstuff and experiment diets

Feedstuff	Diets (100% DM)			
	A	B	C	D
Rice straw ammoniated	40	40	40	40
Rice bran	19	19	19	19
Fresh cassava waste	15	15	15	15
Soybean cake waste	12,50	12,50	12,50	12,50
Coconut meal	12,50	12,50	12,50	12,50
Mineral mix	0,75	0,75	0,75	0,75
Salt	0,25	0,25	0,25	0,25
Fibrolytic enzymes	0	0,75	1,50	2,25
Nutrient Content				
DM (%)	49,10	48,89	49,71	48,59
Crude Fiber (%)	24,04	24,99	24,92	25,30
Crude Protein (%)	11,47	11,35	11,35	11,24
Ethyl Extract (%)	15,91	16,52	17,38	16,67
NFE (%)	43,34	42,37	41,16	41,47
Ash (%)	11,24	10,77	11,19	11,32

Feed offered and refused were weighed and recorded daily to calculate nutrient composition. Rice straw ammoniated was core-sampled and concentrates were grab-sampled and composed for each

treatment for analysis of chemical composition (Lewis et al., 1996). Composite sample were ground to pass through a 1 mm and then analyzed for dry matter (DM), protein, crude fiber, ethyl extract (EE) and ash according to AOAC (1990). The experimental data was subjected to the General Linear Models (GLM) Procedure for orthogonal polynomial contrast analysis of SAS (SAS, 1996) according to a randomized complete block design (RCBD) by using initial body weight as blocks. Significance was shown at  $P<0.05$  unless otherwise noted.

## **Result and Discussion**

The average daily gain (ADG), feed conversion (FC), feed efficiency (FE) and digestibility of dry matter (DM) and organic matter (OM) are presented at Table 2. Statistical analysis showed that adding fibrolytic enzyme to fresh cassava was had no significant effect on gain, ADG, FC, FE and digestibility of DM and OM. This showed that fibrolytic enzyme was not effective to be treated to fresh cassava waste. It was different with founded by several research before. Enzyme mixtures treated to diet containing concentrate diets increased growth rate of feedlot cattle (Boyles et al., 1992 and Beauchemin et al., 1997). Officer (2000) reported that adding fibrolytic enzymes in the diet of ruminants can improve starch, protein and fiber digestibility. Yang et al. (1999) suggested that inconsistent result of enzyme treatment of diets can be due to a number factors, including diet composition, type of enzyme preparation, complementary enzyme activities, amount of enzyme provided, enzyme stability and method of application. Hristov et al. (1998) reported that total tract digestibility's of DM, NDF and CP was not improved when fibrolytic enzyme consisting of cellulose and xylanase added to heifers diets containing rolled barley grain, corn silage and soybean meal. However, Hristov et al. (1998) reported that total tract digestibility's of DM, NDF and CP was not improved when fibrolytic enzyme consisting of cellulose and xylanase added to heifers diets containing rolled barley grain, corn silage and soybean meal. However, Lewis et al. (1996) and Feng et al. (1996) reported increased apparent digestibilities of DM, NDF and ADF in fibrolytic treated grass forage fed to steers.

Table 2. The performance and digestibility of dry matter (DM) and organic matter (OM) of fattening male cattle fed rice straw ammoniated with fresh cassava waste as fermentable energy treated with different level of fibrolytic enzymes

Variables	Diets			
	A	B	C	D
Initial BW (kg)	229,53	236,13	229,75	217,63
Gain BW (kg)	75	70,13	71,75	70,63
Average Daily Gain (ADG, kg)	1,07	1,00	1,02	1,01
Feed conversion (FC)	7,36	8,30	7,67	7,44
Feed Efficiency	13,71	12,68	13,15	13,61
Digestibility (%)				
DM (%)	66,23	62,64	65,30	63,09
OM (%)	71,58	65,97	70,77	68,69

There are many factors influencing efficacy of feed enzyme such as diet composition, microbial source of enzyme activity, mixture of enzyme activities, inclusion rate, heat stability, ambient temperature at feeding and class of animal (Officer, 2000). Rode and Beauchemin (1998) suggested that type of enzyme, activities complement and method of application influence the enzyme effectiveness in ruminant feeds. This is the reason why fibrolytic enzyme treated to fresh cassava waste was not effective to improve performance of fattening local male cattle. This might be caused of chemical composition of fresh cassava waste. According laboratory analysis fresh cassava waste contained 7% of lactic acid. These acids can decrease pH of fresh cassava waste and reducing of pH can impact to activity of fibrolytic enzyme. Therefore, adding of fibrolytic enzyme to this feedstuff was not effective to improve its quality.

Bata (2004) reported that fibrolytic enzyme treated to rice bran can improve performance i.e ADG, FC and FE of fattening weaned male Holstein bull. This differences may be caused by the different of nutrient composition of of those feedstuff.. In addition, physically, fresh cassava waste had high moisture content (76,23%) when treated fibrolytic enzyme, while the moisture content of rice bran was 13%. High moister content will decrease temperature of media where the enzyme will act to hydrolyze cellulose or hemicelluloses in the feedstuff. Reducing temperature will reduce activity of enzyme, therefore, increasing level of fibrolytic enzyme to treat fresh cassava waste was not effective to hydrolyze complex carbohydrate to simple carbohydrate

## Conclusion

It can be concluded that fresh cassava waste can be used as fermentable energy when cattle fed rice straw ammoniated as forage without any treatments.

## References

AOAC. 1990. Official methods of analysis. (13 ed). Association of Official Analytical Chemist, Washington, DC.

Aharoni, Y., A. Arieli, and H. Tagari. 1993. Lactational response of dairy cows to change of degradability of dietary protein and organic matter. *J. Dairy Sci.* 76:3514–3522.

Aldrich, J. M., L. D. Muller, G. A. Varga, and L. C. Griel. 1993. Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. *J. Dairy Sci.* 76:1091–1105.

Arieli, A., Z. Shabi, I. Bruckental, H. Tagari, Y. Aharoni, S. Zamwell, and H. Voet. 1996. Effect of the degradation of organic matter and crude protein on ruminal fermentation in dairy cows. *J. Dairy Sci.* 79:1774–1780.

Bata, M. 2004. The use of fibrolytic enzymes to improve quality of rice bran and cottonseed meal and its effect on nutrient utilization and performance of fattening weaner Holstein bulls in Indonesia

Beauchemin, K.A., S.D.M. Jones, L.M. Rode and V.J.H. Sewalt. 1997. Effect of fibrolytic enzyme in corn or barley diets on performance and carcass characteristics of feedlot cattle. *Can. J. Anim. Sci.* 77:645-653

Boyles, D.W., C.R. Richardson and C.W. Cobb. 1992. Feedlot performance of steers fed steam flaked grain shorgum with added enzymes. *J. Anim. Sci.* 70 (supl.1):133(abstr.)

Feng, P., C.W. Hunt, G.T. Prithchard and W.E. Julien. 1996. Effect of enzyme preparation on in-situ and in-vitro degradation and in-vivo digestive characteristics of mature cool-season grass forage in beef steers. *J. Anim. Sci.* 74: 1349 (abstr.)

Hoover, W. H., and S. R. Stokes. 1991. Balancing carbohydrate and protein for optimum rumen microbial yield. *J. Dairy Sci.* 74:3630–3644.

Hristov, A.N., T.A. McAllister and K.J. Cheng. 1998. Stability of exogenous polysaccharide-degrading enzyme in the rumen. *Anim. Feed Sci. Technol.* 76:165 - 172

Krause, M., K.A. Beauchemin, L.M. Rode, B.I. Farr and P. Norgaard. 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets to growing cattle. *J. Anim. Sci.* 76: 2912-2920

Lewis, G.E., C.W. Hunt, W.K. Sanhez, R.J. Treacher and G.T. Pritchard. 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of forage-based diet fed to beef steers. *J. Anim. Sci.* 74: 3020-3028

Officer, D.J. 2000. Feed enzyme. In: D'Mello (ed). *Farm Animal Metabolism and Nutrition*. CABI Publishing, Wallingford, UK.

Ørskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci. (Camb.)* 92:499-504.

Robinson, P. H., and R. E. McQueen. 1994. Influence of supplemental protein source and feeding frequency on rumen fermentation and performance in dairy cows. *J. Dairy Sci.* 77:1340-1353.

Rode, L.M. and K.A. Beauchemin. 1998. Enzymes to enhance utilization of feed in dairy cow. Research Center, Agriculture and Agri - Food Canada.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. Blauweikel. 1991. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. *J. Dairy Sci.* 74:871-881.

Stokes, S.R., W. H. Hoover, T. K. Miller, and R. Blauweike. 1991. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. *J. Dairy Sci.* 74: 871-881.

Yang, W.Z., K.A. Beauchemin and L.M. Rode. 1999. Effect of enzyme feed additive on extent of digestion and milk production of lactating dairy cows. *J. dairy Sci.* 82:391- 403

SAS® User's Guide: Statistics, Version 5 Edition. 1996. SAS Inst., Inc., Cary, NC.

# **A View of Bogor Climatology Related To the Emerging Anthrax and Avian Influenza Diseases since January 2004 to February 2005: Importance for Early Warning System**

Agik Suprayogi, Heru Setijanto, I Wayan Teguh Wibawan,  
Fadjar Satrija and Widiyanto Dwi Surya

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## **Abstract**

*This study would like to describe that Bogor's climate has a potential emerging tropical animal diseases especially on the emerging anthrax and avian influenza (AI) diseases. Both diseases were frequently outbreak in the region and have a high risk on the public health and also have a significant social-economic impact. Early warning system needs to be developed to anticipate the possible outbreak the diseases. Some climatology parameters data such as air temperature, humidity, rainfall, raining days, soil temperature, wind speed, and sun shine duration were collected since January 2004 to February 2005. Secondary dates from public communication and also from related institution according to the information of outbreak times these diseases were also collected in the periods. This study show that emerging anthrax outbreak was occurred on September to October 2004 and then followed by the emerging AI outbreak on December to June 2005, while it also seen outbreak on January 2004. Anthrax disease well known as a soil borne diseases, the possible reason according to the outbreak might be caused by a changing the climatic condition from dry season (Juni - August 2004) to rainy season (September 2004 - February 2005). Consequence of the climatic changes could remove the spore of *Bacillus anthracis* from the top soil layer to the air by wind speed and also could be possible to spread by flood. Possibility of AI outbreak in the region could be occurred on the lowest air temperature level (25.2-25.8) °C and also highest humidity level (86-90)% rel. condition relatively in month of the years. Related to the biological of AI virus, might be the condition is very favorable weather for the AI virus, unfortunately still lack information in this issue up to now.*

**Key words:** *Climatology, anthrax, avian influenza, Bogor*

## Introduction

Anthrax and Avian Influenza diseases are noted as the strategic animal diseases in Indonesia (Direktorat Jenderal Peternakan, DEPTAN-RI, 1998). Both diseases known as zoonoses have a high risk on the public health and also have a significant socio-economic impact.

Anthrax disease well known as a soil borne diseases, it is caused by bacteria of *Bacillus anthracis*. All mammal, as well as some birds and reptile are susceptible to the bacteria infection. Anthrax, however, is above all an infection of herbivores, especially cattle, horse and small ruminant which ingest the pathogens as spores or vegetative forms from the soil. *B. anthracis* spore apparently survive best in neutral to alkaline soil at a pH level not lower than 6.0, with enough calcium and relatively high nitrogen content. Under suitable conditions (humidity, temperature, pH and content of nutrients), the spores may grow out within the upper layer of the soil and be ingested by the animal with the fodder (Seifert, 1996). *B anthracis* survives in soil a dynamic state in which it undergoes cycles of germination and sporulation dependent on fluctuating conditions in micro-environment (Van Ness, 1971). The vegetative bacilli which are disseminated by sick animals in large amounts from their body openings or which are released from slaughtered or death carcasses sporulate in and at soil at temperature 20 – 32 °C (Seifert, 1996).

Avian Influenza (AI), commonly called “bird flu” is an infectious disease of birds caused by certain types of the influenza viruses that occur naturally among birds. These viruses do not usually infect humans, but several cases of human infection with bird flu viruses have recently been reported. AI viruses are classified into three types, known as types A, B, and C. Influenza viruses infect wide ranges of animals, including humans, birds, pigs, horses, seals, and whales, but their major reservoirs are in wild bird populations. Influenza B and C viruses circulate only among humans, and only A and B viruses cause significant diseases (<http://www.ds-osac.org>, 21.04.2007). The outbreak of AI Type of A (H5N1) in Asia has become a global crisis for animal and human health. On January 27, World Health Organization (WHO), Food and Agricultural Organization (FAO), and the World Organization for Animal Health (OIE) appealed for global collaboration and funding to control the outbreak (Fidler, 2004). The dangers of the diseases is that no one will have immunity to the virus, as no one will have been exposed to it or developed antibodies. Otherwise, no vaccine with guaranteed efficacy can be prepared in advance of such an

outbreak. Human can be infected by respiration, contact (with transfer to eyes, nose or mouth) or ingestion of the virus ([http://www.who.int/water\\_sanitation\\_health](http://www.who.int/water_sanitation_health), last up date 30.05.2006). Up to now there are still lack information related to the environment biological live characteristic of the virus, there is no information according to the comfortable climatic zone for the virus life. Studies on the survival of viruses in human faecal wastes and agricultural animal wastes have indicated that persistence is dependent on several factors, including the virus type, waste type, temperature and other environmental conditions and processes (Sobsey and Meschke, 2003). A study showed that in the animal wastes (feces) AI virus could be longer survive in the high moisture and low temperature condition (Animal Health Australia, Ausvetplan, 2005). This virus is also still infective in the feces for 7 days at 20°C and longer infectivity over 30 days when the declining temperature conducted at 4°C (Webster *et al.*, 1978.). The Influenza virus can survive several weeks on dust, cotton sheets, and glass slides at 22°C (Sobsey and Meschke, 2003), while AI virus reported that the virus still detected in poultry house 2 weeks post-depopulation (Animal Health Australia, Ausvetplan, 2005). In air/aerosols conditions, the virus can survive longer in high relative humidity and low temperature conditions, the other hand the virus can be detected in air samples only to 45 meters downwind of infected flocks (Animal Health Australia, Ausvetplan, 2005). AI virus can also persist for extended periods of time in water depending on the temperature, pH, and salinity ([http://www.who.int/water\\_sanitation\\_health](http://www.who.int/water_sanitation_health), last up date 30.05.2006).

According to the AI outbreaks in Asia including to in Indonesia, H5N1 typically persists in colder temperatures and produces outbreaks during the colder month of the year (Li *et al.*, 2004). However, recent (unpublished) studies mentioned above have shown that current H5N1 strains survive longer in feces at wormer temperature Hulse-Post and Webster, 2005). From the all studies above, the environment biological live characteristic of the AI virus indirectly strake forward to the how important to know the comfortable climatic zone for prevention and control the possible emerging outbreak the disease. It is similar to the Anthrax disease, however it different microorganism and more known advances. Both diseases can outbreak in every where and times especially in tropical countries like Indonesia, because has climatic zone in the forest-humid tropic (Johnson, 1987) more high possibility to grow up well the any microorganism in warm and high humidity climate (comfort conditions for microorganism). For

that reason, early warning system needs to be developed to anticipate the possible outbreak the diseases.

This study would like to describe as a climatic model that Bogor's climate (tropical rain forest zone) has potential emerging tropical animal diseases especially on the emerging anthrax and avian influenza (AI) diseases. This study focused on the climatology data in Bogor especially since January 2004 to February 2005. Both diseases were frequently outbreak in the region and have a high risk on the public health and also have a significant social-economic impact.

## Methods

This study was conducted in Bogor city, West Java-Indonesia has the climate characteristic base on the vegetation available, is hot-humid tropic (Johnson, 1987). Geographically, Bogor is located in  $06.33^{\circ}$  to  $10.9^{\circ}$  latitude south of the equator and  $106.44^{\circ}$  to  $58.5^{\circ}$  longitude east. The latitude is 190 m to 330 m above sea level. Average annual air temperature is  $26^{\circ}\text{C}$  (maximum and minimum temperature are  $30.4^{\circ}\text{C}$  and  $21.8^{\circ}\text{C}$  respectively), average humidity is 70 %rel. with annual rainfall is 3500 to 4000 mm. The wide of Bogor region is approximately 11.850 Ha. The map of Bogor city can be seen in the Figure 1.

### Collecting of parameters

Some climatology parameters data such as air temperature, humidity, rainfall, raining days, soil temperature, wind speed, and duration of sun shine were collected since January 2004 up to February 2005 from Darmaga-Bogor Climatology Station, **Badan Meteorologi Geofisika** (BMG), Republic of Indonesia.



**Figure 1.** Map of Bogor city

## Results and Discussion

Bogor's climate since January 2004 to February 2005 were recorded by Darmaga-Bogor Climatology Station, **Badan Meteorologi dan Geofisika** (BMG), Republic of Indonesia can be seen in the Table 1. A view of climatology in the period reach a pick of air temperature, sun shine duration, and wind speed occurred on April and October 2004 (Both months was  $26.3^{\circ}\text{C}$ ), October 2004 (88 hours), and October 2004 to January 2005 (2.3 km/hours) respectively. These climatic parameters except wind speed started on November 2004 reveal a sharply declining

up to January 2005. Air temperature and sun shine duration on January 2005 dramatically down to be 25.2°C and 35 hours respectively.

Others parameters rainfall, humidity, and raining days reach a lowest level on June to August 2004 (from lowest to highest ranges: 166 to 209 mm/month), on August 2004 (76%rel.), also on August 2004 (6 days) respectively. These parameters will gradually enhance at least up to January-February 2005, reach a maximum level respectively 580 mm/month, 90%rel., and 27 days.

The Bogor's climate since January 2004 to February 2005 recorded can be matched with the evidence of the diseases outbreak in Bogor or around this city as an attempt to know the characteristic of climate related to the disease outbreak. The information would be very important to be used as an early warning system to prevent and control the possible occurrence of disease outbreak. The outbreak occurrence of both diseases can be seen in Table 2. This study show that emerging anthrax outbreak was occurred on September up to October 2004 and then followed by the emerging AI outbreak on January up to March 2005, while it also seen outbreak on January 2004.

Anthrax disease well known as a soil borne diseases (Seifert, 1987), the possible reason according to the outbreak might be caused by a changing the climatic condition from dry season (June-August 2004) to rainy season (September 2004-February 2005). It could be seen that on the dry season the rainfall, raining days, and humidity relatively take in the low level was (166-209) mm/month, (6-15) raining day/month, and (76-83) % rel respectively. On September, beginning to be rainy season the all climatic parameters above dramatically increase and simultaneously followed by increasing the wind speed and air temperature significantly was (2.2-2.3) km/hours and (25.8-26.3)°C respectively. The climatic condition could remove the spore of *bacillus anthracis* from the top soil layer to the air by wind speed and also could be possible distributed by flood, and might be possible *B. anthracis* spore apparently has under suitable conditions (humidity, temperature, pH and content of nutrients) for very well survive and growing up.

The emerging of AI outbreak occurred on December 2004 up to June 2005, which affect to the 3 peoples suffering and eventually 2 of them are death in Tangerang (<http://www.ds-osac.org>, accessed on 21.04. 2007). Actually, the AI outbreak in all Indonesia regions had been beginning one year before, on December 2003 to January 2004 in the poultry (Departemen Pertanian-RI, 2004). It was also strengthened by WHO, FAO, and OIE) which declared that AI outbreak in Asia on 27 January 2004 (Fidler, 2004).

Table 1. Bogor's climate since January 2004 to February 2005

Month in 2004- 2005	Air Temp. (°C)	Humidity (%rel.)	Rainfall (mm)	Raining day (days)	Wind Speed (km/hours)	Sun Shine Duration (hours)
Jnr-04	25.6	88	404	26	2.1	57
Feb-04	25.3	89	327	28	1.8	30
Mrc-04	25.8	85	432	24	2.3	53
Apr-04	26.3	87	640	25	2.3	76
Mei-04	26.1	86	374	23	1.7	70
Jne-04	25.4	82	169	13	1.8	75
Jly-04	25.5	83	209	15	1.7	79
Aug-04	25.7	76	166	6	2.1	87
Spt-04	25.8	82	392	22	2.2	82
Oct-04	26.3	80	277	21	2.3	88
Nvb-04	26.1	86	401	27	2.3	64
Dsb-04	25.8	86	432	25	2.3	35
Jnr-05	25.2	90	537	27	2.3	35
Feb-05	25.4	89	580	25	2.1	48

Source: Darmaga-Bogor Climatology Station, **Badan Meteorologi dan Geofisika (BMG)**, Republic of Indonesia.

The possible reason for both AI outbreak occurrences in the periods might be the condition is a comfortable climate for the biological of AI virus in air temperature and humidity. In this time (December 2004 to February 2005), average of the sun shine duration and air temperature sharply decreased up to low level were (35-48) hours/month and (25.2-25.8) °C respectively. Simultaneously the average of rainfall, raining days, and humidity enhance up to (432-580) mm/month, (25-27) days/month, and (86-90) %rel. respectively.

This study presents a strong tendency that possibility of AI outbreak in the region could be occurred on the lowest air temperature and also highest humidity relatively in month of the years. This finding seem too similar with Li *et al.* (2004) reported that H5N1 typically persists in colder temperatures and produces outbreaks during the colder month of the year. Besides, a study showed that in the animal wastes AI virus could be longer survival in the high moisture and low temperature condition (Animal Health Australia-Ausvetplan, 2005). Unfortunately, the comfortable climatic zone for biological AI virus has no known exactly up to now, however several studies have already addressed to the persistence of the virus in some medium (water, bird, feces, dust, and air) related to the influence of its environment.

Table 2. The outbreak occurrence of Anthrax and Avian Influenza diseases since January 2004 to February 2005

Month in 2004-2005	Occurrence of Diseases Outbreak		Remarks and References
	Anthrax	Avian Influenza	
Jnr-04	NI	<i>Outbreak</i>	Dirjen Bina Produksi Peternakan said that from December 2003 to January 2004, 4.7 million poultries in all Indonesian region suffered by AI disease (Departemen Pertanian-RI, 2004).  On 27 January 2004, WHO, FAO, and OIE declared that AI (H5N1) outbreak in Asia (Fidler, 2004).
Feb-04 to Aug-04	NI	NI	-
Spt-04	<i>Outbreak</i>	NI	24 October 2004, Ministry of Agriculture, Republic of Indonesia (RI) declared that 6 peoples death after consuming meat of goat in Babakan Madang-Bogor caused by Anthrax disease (Badan Penelitian dan Pengembangan, Deptan. IAARD.Online, 27 October 2004).
Oct-04	<i>Outbreak</i>	NI	Head of R&D, Dept. of Agric.-RI said that anthrax disease always emerge every years in endemic region, Bogor (Cakrawala, Pikiran Rakyat, 28 October 2004).  The emerging of Anthrax cases in Bogor district occurred on September 2004 (2 peoples suffered) and continued to October 2004 (30 peoples suffered, 6 peoples of them death) (Wulantari, 2006).
Nvb-04	NI	NI	-
Dsb-04	NI	<i>Outbreak</i>	From December 2004 to June 2005 confirmed that 3 people suffered by AI virus (H5N1), 2 of them death in Tangerang (near border of Bogor) ( <a href="http://www.ds-osac.org">http://www.ds-osac.org</a> , accessed on 21.04. 2007).
Jnr-05	NI	<i>Outbreak</i>	
Feb-05	NI	<i>Outbreak</i>	

NI.: No Information

Both the emerging of anthrax and AI diseases outbreak, besides affected by Bogor's climatic condition like in mentioned above. It is really possible also be influenced by physiological status of the animal (sheep) in the tropical rain forest environment showed the extremely suffering by humidity stress, therefore respiration rate abnormality could be occurred. This microclimate condition indicate the uncomfortable for enrichment productivity and health on the animal production system (Suprayogi and Astuti, 2006).

## Conclusions

The Bogor's climate has potential emerging tropical animal diseases especially on the emerging anthrax and avian influenza (AI) diseases. It could be supposed that the Bogor's climate characteristic tend to lead to be warm temperature, high rainfall, and high humidity. The condition is very favorable weather for biological AI virus life, and germination and sporulation of *B. anthracis* in the soil. This study show that emerging anthrax outbreak was occurred on September up to October 2004 and then followed by the emerging AI outbreak on December 2004 to February 2005, while it also seen outbreak on January 2004. The possible reason according to the anthrax outbreak might be caused by a changing the climatic condition from dry season (Juni-August 2004) to rainy season (September 2004-February 2005). Possibility of AI outbreak in the region could be occurred on the lowest air temperature (25.2-25.8)°C and also highest humidity (86-90)%rel. condition relatively in month of the years. Unfortunately, there was no exact information according to the comfortable climatic zone for biological AI virus. The information of when the possibility of diseases outbreak will be, it would be very important to be used as an early warning system to prevent and control the possible occurrence of disease outbreak.

## References

Animal Health Australia, Ausvetplan, 2005. Avian influenza (version 3.1). Australian Veterinary Emergency Plan (AUSVETPLAN), edition 3, Primary Industries Ministerial Council, Canberra, ACT.

Badan Penelitian dan Pengembangan, Deptan. IAARD.Online. Berita utama: Bogor terserang antrak, 27 October 2004. (humas @litbang.deptan.go.id)

Cakrawala, Suplement *Pikiran Rakyat* khusus IPTEK, Berita Utama: Penyakit Anthrax muncul di daerah endemis , 28 October 2004.

Departemen Pertanian-RI. 2004. Isu Nasional Pembangunan Pertanian (Periode 26-28 Januari 2004, No. 01/2004): Flu Burung. Biro Hukum dan hubungan masyarakat-Deptan-RI.

Direktorat Jendral, Departemen Pertanian, Republik Indonesia. 1998. Keputusan Direktur Jendral Peternakan No. 103/TN.510/KPTS/DJP/0398, tentang jenis-jenis penyakit hewan menular yang mendapat prioritas pengendaliannya.

Fidler D.P., 2004. Global outbreak of Avian Influenza A. (H5N1) and international law. The American Society of International Law (ASIL) Insights. <http://www.asil.org/insights>, (accessed 21.04.2007).

Johnson H.D. 1987. Bioclimatology and The Adaptation of Livestock. Elsevier, Amsterdam-Oxford, New York, Tokyo.

Li K.S, Guan Y., Wang J., Smith G.J.D., Duan L., Rahardjo A.P., Puthavanthana P., Buranathai C., Nguyen T.D., Estoepangestie A.T.S., Chaisingh A., Auewarakul P., Long H.T., Hanh N.T.H., Webby R.J., Poon L.L.M., Chen H., Shortridge K.F., Yuen K.Y., Webster R.G., and Peiris J.S.M.. 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature*, 430:209-308.

Oversease Security Advisory Council (OSAC): Avian Influenza in ASIA. [Http://www.ds-osac.org](http://www.ds-osac.org), (accessed 21.04.2007)

Seifert H.S.H. 1996. Tropical Animal Health. Kluwer Academic Publishers. Dordrecht, Boston, London.

Sobsey M.D. and Meschke J.S., 2003. Virus survival in the environment with special attention to survival in sewage droplets and other environmental media of fecal or respiratory origin. Paper for WHO meeting (International SARS Symposium) held on 23-25 September 2003 in Rome, Italy.

Suprayogi A., Astuti D.A. 2006. Physiological status of indoor sheep in the tropical forest (HPGW) environment. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, University of Kassel-Germany. Beiheft 88:1-6.

Van Ness G.B., 1971. Ecology of anthrax. *Sc. 172*, 1303-1307.

Webster R.G., Yakhno M., Hinshaw V.S., Bean W.J. and Murti K.G. 1978. Intestinal influenza: Replication and characterization of influenza viruses in ducks. *Virology*, 84(2):268-278

World Health Organization (WHO). Review of latest available evidence on potential transmission of avian influenza (H5N1) through water and sewage and ways to reduce the risks to human health. [Http://www.who.int/water\\_sanitation\\_health](http://www.who.int/water_sanitation_health), (last update 30.05.2006, accessed 21.04.2007).

Wulantari E. 2006. Makalah Lokakarya, Dinas Kesehatan Kabupaten Bogor: SKD and Public Awareness Dalam Rangka Pengendalian Penyakit Anthrax pada Manusia. Departement Ilmu Penyakit Hewan dan Kesehatan Masyarakat, Fakultas Kedokteran Hewan, Institut Pertanian Bogor.

## **Alternative Utilization of Storage Roots Flour of Yam Bean (*P.erosus*) in Wheat Flour-Based Food Products (Bread)**

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### **Abstracts**

*Like many countries in Asia, Indonesia use significant number of foreign exchange to import large quantities of wheat flour in order to satisfy the rapid growing domestic demand of wheat flour-based food products. At the same time many of these countries including Indonesia also produce different types of root and tuber crops such as cassava, sweet potato and yam bean that could be used to replace certain amounts of wheat flour in different food products. This research tries to explore the possibility of incorporating sun-dried storage roots flour of yam bean in making bread. Yam bean storage roots were sliced, sun dried and grounded to obtain yam bean storage roots flour. Composite flour was made by replacing 5, 10, 20, and 40% of wheat flour with yam bean storage roots flour. Results showed that substitution up to 20% of the wheat flour is possible to obtain bread with satisfactory properties. However, 10% substitution seems to be better for all sensorial quality attributes. The higher the substitution, the sweeter the product as indicated by the sweetness perception of the panelists. This finding indicates that it is not only technically feasible, but also economically attractive to use yam bean storage roots flour in preparing wheat flour-based food products (bread) with side effect of generating and broaden its additional uses as well as reducing wheat flour import.*

*Key words:* Yam bean storage roots flour, bread, and sensorial quality.

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## Introduction

Processing of agricultural products increase their economical value and makes an important contribution to agricultural development and farm income. Such effort ensures income and employment for many peoples. Although it remains minor crop, yam beans (*Pachyrhizus spp.*) nowadays are grown by many farmers in Indonesia as alternative cash crop for the local market. Karuniawan (2004) reported that the demand for the storage roots are steadily increasing because currently they have been used not only as food for direct consumption but also as processed-food such as ‘asinan bengkuang’ (salted sliced storage roots), ‘manisan bengkuang’ (candied sliced storage roots), yam bean syrup and juice. Moreover, non-food uses also has been developed such as yam bean face tonic, yam bean masker, and yam bean powder (bedak dingin) which are mostly used by woman in Indonesia as cosmetics. By processing yam bean storage roots into new food products, the value of this commodity can be enhanced so that it can result in higher and more stable market prices which will benefit for farm producers. For many value-added uses, root and storage roots crops such as yam bean must compete against maize, wheat or other sources of raw materials. In addition, yam bean, due to its high water content, may require additional conditionings such as chipping and drying to turn them into forms that are more easily storable and transportable.

High amount of scarce foreign exchange has been used by Indonesia government to import large quantities of wheat flour to satisfy the rapidly growing demand for bread and other wheat flour-base products for the expending population.

Since post-harvest utilization of the crop as food remains limited, this research is aimed to study the possibility of using sun dried materials of yam bean storage roots as composite flour to make wheat flour-base food products (bread).

## Materials and Methods

### Materials

Harvested storage roots of yam bean *P.erosus* originally from Java island which were grown in Bogor – Indonesia were peeled and washed several times using clean water before sliced and sun-dried for several days. The dried storage roots were ground using a Laboratory mill 120 (Perten Instruments AB, Huding, Sweden) through a 60 mesh

screen and stored in airtight plastic bottles until used. Wheat flour (Type 405 produced for Penny Markt GmbH – Köln) was used as standard flour. All other ingredients used in preparing of bread were of standardized quality materials for commercial baking obtained from local supermarket.

### **Methods**

Fine flour obtained from yam bean storage roots was used to substitute certain quantity of wheat flour for baking purposes. Composite flour containing 5, 10, 20, and 40% of yam bean storage roots flour and wheat flour were prepared and used for bread preparation. Panel test (Garber et al. 2003, Stone and Sidel 1993, Chin-Lin Hsu et al. 2004, Kihlberg et al. 2005) was performed to get information (panelist's perception) on sensorial quality attributes such as aroma, texture, taste, hardness, adhesiveness (mouth feeling), elasticity and sweetness of the three products. The panelists were asked to determine based on their own perception all the defined quality attributes in comparison to the standard product (no substitution with yam bean flour) of related product and between the treated products itself, by filling a provided hedonic scale card. The panelists were required to rinse their mouth by drinking water between each sample. Results obtained were considered accepted or rejected when it gain more than 50% panelist perception. Sensorial qualities of aroma, texture, taste, and sweetness were measured using a hedonic scale with the following scoring criteria: *very bad* (1), *bad* (2), *slightly bad* (3) representing **dislike** or **rejected** products (low quality), and **accepted** products are those consider *neutral* (4) or medium quality; *good* (6) and *very good* (7) as high quality products. In terms of sweetness, *too weak* (1), *weak* (2) and *too strong* are considered as low quality products which are **rejected**.

Substitution of wheat flour with 5, 10, 20 and 40% of yam bean storage roots flour were applied in bread preparation. In this experiment, normal bread procedure (ICC-Standard No.131) was applied on the basis of 500 g wheat flour for the calculation of the substitution and the addition of other components such as yeast (5%), salt (1.5%), sugar (1%), margarine (1%) and amount of water which was calculated based on water absorption, measured separately using Valorigraph (similar to Farinograph) based on ICC Standard Method No. 115/1. All these ingredients were of standardized quality for commercial baking, purchased from a local food market in Göttingen. Well-mixed composite flour was weighted in the mixing container of

kneading machine and simultaneously combines with all other ingredients in this bowl and let the bread machine mixing and kneading it for 10 minutes, and then stops (moulding and proofing) for 30 minutes and kneading again for 10 minutes. Once it was ready, the dough was transferred into a baking pan, covered with a clean dish towel and let it proof at a warm place (35°C) for 30 minutes continued with baking at preheated oven at 250° C for 40 minutes, or until the loaf surface / crust turn into lightly brown. During baking, the surface of the loaf was sprayed with water three times. The loaves were taken out of the tins, cooled at room temperature for 2 hours and then sliced. As much as 19 peoples who are mostly regular users of bread were chosen to be the panelist. The composition of the panelists are quite balance, consist of 1 man and 1 woman who were in the age range of 50 – 60 years old, 6 men and 6 women in age between 30 – 40 and 3 women and 2 men of 20-30 years old.

## Results and Discussion

In the following figures, panelist perceptions of aroma, texture, taste, sweetness, and elasticity of bread of each treatment were presented in simple bar chart to show percentage of panelist who chooses certain sensorial quality category of the related hedonic scale.

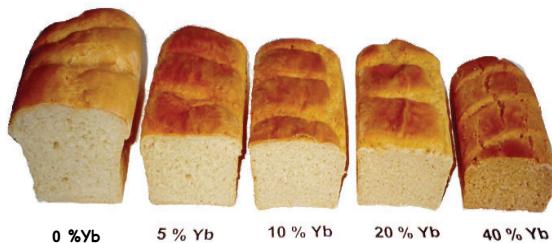


Figure 1. Comparison of bread without yam bean (Yb) and breads Containing yam bean storage roots flour in different portion

Panel test result showed that most the panelists rejected the aroma of bread containing 40% yam bean storage roots flour (Figure 1). 26% of the panelists consider the bread aroma as slightly bad, bad

(42%) and very bad (5%). Statistically, the highest substitution (40%) have significantly different aroma compared to the other lower replacement products. The same bread (40%) was also found having rejected texture (73%) and taste (69%) (Figure 2 and 3) which were also significantly different to lower replacement products for the above quality attributes (Table 1).

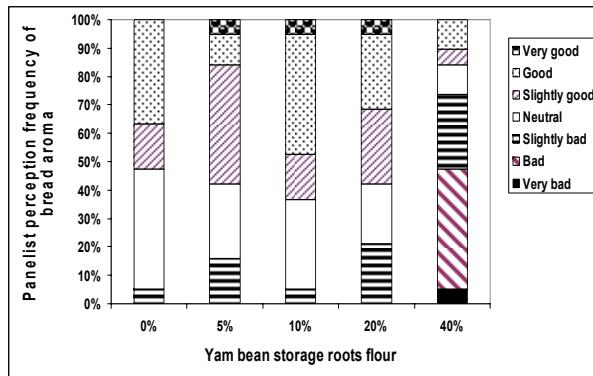


Figure 2. Panelist perception of aroma of bread made of composite flour of yam bean storage roots and wheat flour

Table 1. Sensorial evaluation of yam bean storage roots (Yb) flour incorporated breads

Bread type (%Yb) §	Aroma *	Texture *	Taste* Sweet ness*	Hardness* Adhesi veness	Elasticity * Elasticity *
0	4,8 a	5,3 a	4,9 a	2,7 c	1,2 c
5	4,6 a	4,8 ab	4,2 ab	2,7 c	1,2 c
10	5,1 a	5,1 ab	4,7 a	2,7 c	1,9 ab
20	4,7 a	4,7 b	4,6 a	3,2 b	1,7 b
40	3,0 b	3,8 c	3,4 b	4,0 a	2,2 a
LSD: a = 0,05	0,76	0,54	0,99	0,46	0,36
				0,46	0,28

\* Values followed by the same letter in the same column are not significantly different (P<0,05)

§ Percentage of wheat flour replaced by yam bean storage roots (Yb) flour in the formulation

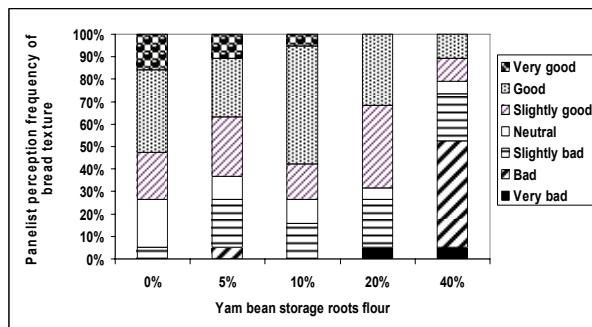


Figure 2. Panelists' perception of texture of bread made of composite flour of yam bean storage roots and wheat flour

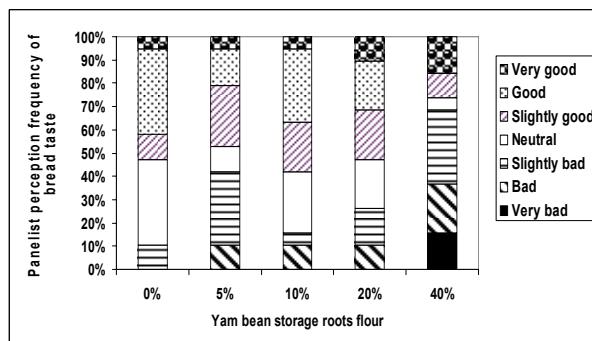


Figure 3. Taste perception of bread made of composite flour of yam bean storage roots and wheat flour

In terms of sweetness (Figure 4), bread having 40% yam bean flour was considered by 32% panelists as rejected product because the sweetness was too strong but much more panelist (68%) accepted this product.

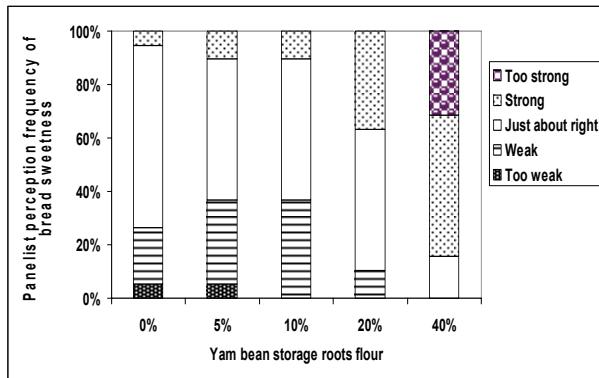


Figure 4. Sweetness perception of bread made of composite flour of yam bean storage roots and wheat flour

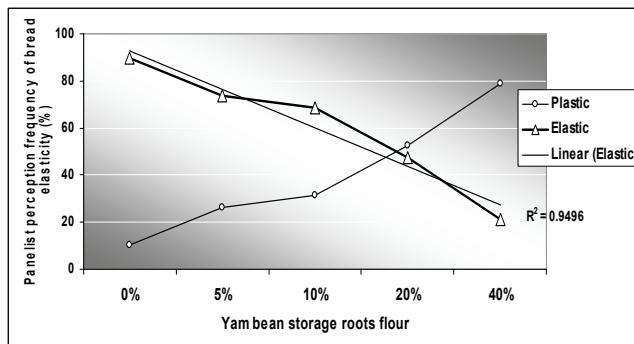


Figure 5. Elasticity characters of bread made of composite flour of yam bean storage roots and wheat flour

Positive correlation was detected between elasticity and portion of yam bean storage roots flour incorporated (Figure 5). The higher the amount of yam bean flour used, the lower the elasticity, but the plasticity increase linearly following the substitution. There were no significant differences of mouth feeling (adhesiveness) of all bread tested (Table 1).

## Conclusion

Sun-dried flour of yam bean (*P.erosus*) storage roots could be used as much as 20% to substitute wheat flour in bread preparation process. However, 40% substitution gave better sweetness and accepted by majority of the panelists although the taste and aroma were rejected. Elasticity of bread decrease as the substitution ratio increase. As various ingredients can be included in bread preparation to improve bread quality (Chen and Chiang 1984), research to improve yam bean flour is recommended in order to enhance and diversify the use of yam bean storage roots materials in food products.

## References

Chin - Lin Hsu, Shu - Lin Hurang, Wenlung Chen, Yih - Ming Weng and Chin - Yin Tseng (2004). Qualities and antioxidant properties of bread as affected by the incorporation of yam flour in the formulation. International Journal of Food Science and technology. 39, 231-238. Blackwell Publishing Ltd.

Chen K. L. and Chiang W.C. (1984). Manufacture and quality improvement of composite bread made from sweet potato flour and wheat flour. Food Science, 11, 66-77.

Garber Jr., Lawrence L., Hyatt E.M., Richard G. Starr Jr. (2003). Measuring consumer response to food products. Food Quality and Preference 14 (2003) 3-15. Elsevier Science Ltd.

Stone H. and Sidel J.L. (1993). Sensorial evaluation practices. Academic press Limited 24 – 28 Oval Road, London NW1 7DX.

ICC Standards (1999). Standard Methods of the International Association for Cereal Science and Technology (ICC), Vienna, Austria

Karuniawan A. (2004). Cultivation status and genetic diversity of yam bean (*Pachyrhizus erosus* (L.) Urban in Indonesia. Doctoral dissertation – Georg-August University Göttingen – Germany.

Kihlberg Iwona, Listbeth Johansson, Oyvind Langsrud and Einar Røsvik (2005). Effect of information on liking of bread. Food Quality and Preference 16 (2005) 25-35. Elsevier Ltd. Available online at: [www.sciencedirect.com](http://www.sciencedirect.com)

## **Comparation Study Progress On Anoa's Behaviour Prior To Conservation Program**

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### **Abstract**

*Anoa is small buffalo-like animals endemic to the Indonesian island of Sulawesi. This species is vulnerable due to its restricted range and the possibility of extinction from several threats including hunting, habitat loss and possible natural catastrophes. The management and conservation objective is to maintain genetically viable, self-sustaining, free-living Anoa population(s). In order to achieve this goal, it is necessary to understand its behavior both in its in-situ habitat as well in the ex-situ area. The in-situ study area was took place in Lore Lindu National Park of Central Sulawesi which was compared to the report from ex-situ observation of Taman Safari Indonesia Bogor. The behavioral data were collected by direct and indirect observations during wet season. Some references were used to obtain the conclusion of comparison study report. The observations were partly undertaken in the feeding grounds and in the wallowing sites. It can be observed that Anoa could become very aggressive during the breeding seasons. Generally, there are no significantly difference behaviors in both observed habitat of Anoa.*

**Keywords:** Anoa, in-situ, ex-situ, behavior

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## Introduction

Anoas are the smallest of the extant wild cattle species. They are stocky, short limbed and thick necked. Young Anoas have a thick covering of yellowish brown woolly hair. Adults are more variable in color than juveniles but are predominantly brown or black. Males are usually darker than females. Both male and female Anoas have horns (as in all other extant Bovini). Both species are reputed to have an exceptionally thick hide.

Little is actually known about the habitat preferences of the Anoas. Both species seem to require forested areas. *Bubalus depressicornis* is reported to inhabit swampy areas within lowland forest and was reportedly common along coasts in the past while *B. quarlesi* is reported to occur in montane forest up to 2000 m above sea level (NRC, 1983). However, the mountain anoa is sometimes found at sea level and the lowland species is sometimes found at high elevations in mountainous areas (MacKinnon and MacKinnon, 1979). Like other wild buffalo the anoas require sufficient water for wallowing and bathing. It is probable that mineral springs (or licks) are also required.

Anoas have been reported by some local people to prefer well drained and rugged areas without dense undergrowth when feeding and relatively open and dry, ridge-top areas when resting and ruminating. Although the other observed that the mountain anoa prefers dense forest over more open sub-alpine vegetation.

Anoas have a varied diet and are known to eat grasses and other herbs, aquatic plants, the leaves of shrubs and young trees, bark and fruit. In addition, they have been recorded to drink sea water which is thought to fulfill their mineral needs in areas that do not have salt licks or mineral spring water. Captive anoas are fed a diet of hay and herbivore pellets. (Parker, 1990; Nowak, 1999; Anonymous, 2001; Massicot, 2001). During dry season, *Bubalus sp.* was increasing its variation of feed by assorted from plant and alternative crops.

Pujaningsih (2005) reported that Anoa (*Bubalus sp.*) has large variation of forage crop which wide enough and well adapted by alternative feed – both processed and or not processed. Eleven type of vegetation was founded and known as delicacies feed for Anoa, namely, *Areca sp.*, *Elatostema sp.*, *Rubus sp.*, *Zingiber sp.*, *Nephrolepis sp.*, *Cyrtandra sp.*, *Begonia sp.*, *Eragrostis sp.*, *Saccharum sp* and also two types of crop by the local's name of Kaloma (Fam.Palmaceae) and Padalebo (Fam.Utricaceae) which has not been identified by its genus and also its species yet. *Areca sp* (Gompu), *Elatostema sp* (Leluha),

*Zingiber sp* (Karondowana/Katimba) and *Cyrtandra sp* (Utawana) represent as the dominant and favorite vegetation for Anoa. Moreover, *Elatostema sp* was founded in a large of amount on the middle edge of National Lore Lindu Park. *Rubus sp*, *Zingiber sp*, *Nephrolepis sp*, *Begonia sp* and Kaloma were classified to rarely seen in the field research

Most reported sightings of anoas are lone animals or pairs, although a group of five ran past an expedition climbing Mount Nokilalaki in Central Sulawesi. Some states that anoas are shy and largely nocturnal, hiding in dense undergrowth during the day but this may be a response to disturbance by humans –particularly hunting.

Anoas have never been domesticated but their meat, horns and hide are valued throughout Sulawesi. They tend to avoid settlements and other frequently used areas and consequently damage caused by them is minimal. Despite their aggressive and nervous temperament it has been suggested that anoas might make potentially valuable livestock animals. It has also been suggested that the offspring of an anoa x water buffalo cross could produce a useful stock animal. Reported also by local people that captured anoas are often kept as pets and that young anoas are sometimes reared by domestic water buffalo. However, Whitten *et al.*, (1987) remark that while anoas were caught by the Toraja people, who attempted to breed them for meat, their aggressive nature even after several years in captivity meant that they were not used as domestic animals.

In contradiction of these statements, an observation has been done in Ragunan Zoo in 1973 which reported that Anoa could recognize and adapt to their keeper. They were freely following their keeper into their cages. Refer to this information, the study was aimed to make a comparation of anoa's behavior both in *ex-situ* and *in-situ*.

## Method

Information from Taman Safari Indonesia (TSI) Bogor about lowland anoa's behavior was collected because of the model management in this area close to the real habitat of the animal. It will compare to the anoa's behavior in Lore Lindu National Park. Refer to Malih (1972), anoa from Central Sulawesi was hybrid from Anoa of North Sulawesi and Anoa of South Sulawesi, so it can be predicted that Anoa's behavior from Central Sulawesi close to both anoas' type from both areas.

Data of lowland anoa's behavior were collected by using literatures and references which were combined with local information given by local people. Collected data and information divided into two discussions. First is social organization and general behavior. Second is about feeding behavior.

## Discussion

### *Social organization and general behavior*

It was informed by the local people that Anoa tracks are often found but the animal itself is seldom seen. During the day they hide in dense undergrowth, while at night they move down to the alang-alang grasslands near the sea in search of water and salt. It is thought that the nocturnal habit of anoa is in response to the hunting pressure by man, but it is relative, because refer to Grzimek (1972) cited by Jahja (1996) the wild water buffalo (*Bubalus bubalis*) is known to graze predominantly at night and spend the day resting and wallowing in the mud. In the daytime, the animals are active mainly in the morning between 06.00-09.00 am, and in the afternoon, after 04.00 pm.

In *ex-situ* area, the daily activity of the animals was also observed to compare it with that of the animals in the wild. Feeding activity was high from 07.00 – 09.00 am, and from 06.00-08.00 pm. The intensity of feeding activity in the first period was influenced by the food supply at 07.00 am. In the second period of feeding activity, however, they fed on the rest of their morning food.

Unlike all other wild cattle, the anoa is seldom seen in groups, all usually alone or in pairs (Grzimek, 1993). Mustari (1995) reports 40 observations of which 21 were of single adult males, 3 were of single adult females, 9 were of an adult male with an adult female, 5 were of adult females with infants and 2 were of an adult female, a sub adult male and an infant. No groups larger than three were encountered – however the estimated population size in his study area (Tanjung Amolengu, South-East Sulawesi) was only 8-12 animals. In TSI anoa make their own group with the same average age. Local people from Lore Lindu National Park reported about 30 animals in a group around their springs. The lowland anoas are monogamous during the mating season. Adult males have not observed to consort with more than one adult female during any particular breeding season, though it may choose another in a subsequent breeding season.

Like other wild buffalo the anoas wallow and bathe in pools of water and/or mud but it is not known whether such activities are

essential to their well-being. It is probable that mineral springs or licks are also required. Anoa also reported by Whitten *et al.*, (1987) and Mustari (1995) like to drink seawater which might fulfill their mineral needs in areas without licks or springs. Social licking was one of the behaviors of the animals in the zoo. Either the male was licking on the female or conversely. Licking the head, neck, backsides, abdomen and the genital regions was common.

When in danger, anoas flee by leaping, if humans approach within critical distance, it does not flee, instead it attacks violently. The anoa is considered very excitable and dangerous to its opponent since its sharp horns can be used like daggers (Grzimek, 1993). There was some information that anoa is dangerous and has been known to attack and wound people (Sugiri, 1990). Many people in TSI know about this aggressive animal. There was many times attacked people when he escaped from his cage or before he stayed outside (not in the cage), when the keeper did not put them yet into his cage. Besides attack peoples, many anoas attacked young antelope such as young nilgais, timor deers, sambar deers or elands. They horned them as a toy and dragged them violently.

It was reported by Fajar (1973) that anoas in Ragunan Jakarta Zoo have a good relationship with their keeper. Although in the breeding season, the keeper must be more carefully to face the aggressiveness of this dwarf buffalo. Popenol (1983) observed also a good relationship between anoa and the keeper in Berlin zoo. Furthermore it has been recommended by Popenol (1983) although somewhat aggressive and pugnacious, anoas might make a suitable livestock animal. They grow and reproduce well in captivity and are adaptable and intelligent. Their small size makes them easier to handle than many other wild bovines. They can be tamed enough to obey commands ("lie down," for example) but they remained nervous and likely to butt strangers.

### ***Feeding behavior***

Anoas at TSI eat 10 kg concentrate and 60-70 kg fresh king grass per day, with carrot as a supplement diet twice a week given 20 kg per each Anoa. Anoa has been reported to require a mineral rich diet. TSI uses mineral block as a mineral supply hanged every day in every cage they put in. Some of them usually mess up the grasses which are fed with the horn before they eat it. They eat together at the same time twice a day, some of them sometimes dominated with another, but it

happened for a while. Dominated from one or two anoas when they are fed has been reported by TSI's keeper, but it took place sometimes.

The foods were given to the animals at 07.00 am. The animals fed first on the vegetables, especially *Ipomoea aqua*, *Arachis hypogea* and *Cucurbita sp* then on the other foods. The animals did not eat all the food at once, but on many occasions. In contrast to the diet of anoas living in the reserve, in the zoo, the diet is mostly vegetables and fruits. The diet diversity is higher in the natural habitat than in captivity. Anoa obtains minerals from springs and other vegetation. Their tracks were frequently found around the springs and along the lake shore. Local people also observed adult anoa drinking sea water.

The energy metabolism and heat production of free-ranging animals is highly variable, depending on the activity of the animal, is diet, thermoregularly function, sex, reproductive condition, time of day and year, hair or feather characteristics, weather factors, parasites and pathogens and various social and psychological effects. In the natural habitat, anoas need more food and more energy than those in the zoo.

The anoa is very sensitive to human presence and is intolerant of domestic livestock. Wallace (1898) cited by Jahja (1996) mentioned that the anoa never inhabits places where there are deer. This is probably a matter of competition as anoas are more browsers and eat only the young leaves or tips of grass leaves while deer are more intensive grazers

As a browser, anoas consume more food to obtain a certain quantity of protein in their diet. They have to consume more food than the animals whose diet has higher protein content e.g. the domestic animals which habitually feed on the diet have high protein content.

## Conclusion

- There are no significantly anoa's behavior differences between *ex-situ* and *in-situ* area.
- Anoa might make as a suitable livestock animal.
- The use of cage (e.g. Zoo) is more effective to domesticate Anoa compare to free-range model e.g. Taman Safari Indonesia, Bogor

## References

Fadjar, FS. 1973. Anoa di Kebon Binatang Ragunan Jakarta. Universitas Nasional Jakarta.

Grzimek, B. 1968. Grzimek's Tierleben. Enzyklopaedia der Tierreiches. XIII. Säugetiere 4. Kindler Verlag. Zuerich. 379-491 pp.

Jahja, MM. 1996. The possibility of breeding anoa in captivity: An alternative for conservation of the species. Department of Forestry, Fac. of Agriculture, Hasanuddin University, Ujung Pandang, Indonesia.

Malih, IDG. 1972. Laporan Permulaan Penelitian Systematik Sapi Cebol dari Sulawesi di Kebun Binatang Surabaya.

Malik, A., Pujaningsih, R.I. and Labiro, E. 2004. Participatory of Wildlife Conservation in Central Sulawesi Indonesia. (*A Review on a Case study of Anoa Bubalus sp. as one of endemic endangered animal from Sulawesi, Indonesia*). In: Proceeding of The 5th International Symposium-cum-Workshop "The Role of German Alumni in Rural/Regional Development and Entrepreneurship " by SEAG, Royal Agricultural University and the University Consortium Georg-August-University Göttingen, University of Kassel and Philipps University Marburg. Phnom Penh, August 23 - 27, 2004.

Mustari, AH. 1995. Population and Behavior of Lowland Anoa (*Bubalus depressicornis* Smith) in Tanjung Amolengu Wildlife Reserve South-East Sulawesi, Indonesia. Georg - August University, Goettingen, Germany. (Thesis).

Mackinnon, J and K. Mackinnon. 1979. Animals of Asia. The Ecology of the Oriental Region. Peter Lowe, London

Popenol, H. 1983. Little-known Asian Animals with a Promising Economic Future. Managing Tropical Animal Resources, Natural Research Council, Washington DC. Nat.AC.Press. 64-68 pp.

Pujaningsih, R.I., Sukamto, B dan Labiro, E. 2005. Identification and Feed Technology Processing for Roughage in Term of Anoa (*Bubalus* sp) conservation. National Seminar of Fundamental Research. Jakarta, 16-18<sup>th</sup> May 2005

Pujaningsih, R.I. 2005. Identifikasi Vegetasi Pakan Alami Anoa (*Bubalus* sp). In: Proceeding of the 6th International Symposium-cum-Workshop "(In-) Equity and Development: the Role of Science and Technology " by SEAG, Gadjah Mada University and the University Consortium Georg-August-

University Göttingen, University of Kassel and Philipps University Marburg, Yogyakarta, August 22 - 26, 2005

Sugiri, Nawangsari. 1990. The systematics and the captive breeding of Anoa from Indonesia: Faculty of Veterinary Medicine & Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor.

Whitten, A., M., Mustafa and G.S. Henderson. 1987. The ecology of Sulawesi. Gadjah Mada University Press, Yogyakarta. 38-41; 412-414; 539-540 pp

## Rabies Case Study On Dog's Head (*Canis Familiaris*) In Manado, Airmadidi & Langowan Wet Markets

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### Abstract

*Rabies is mainly caused by the Lyssa virus, Rhabdoviridae family, known as “penyakit anjing gila” (Indonesia), “Tollwut” (Germany), “La Rage” (French) and it is a zoonotic disease. Dogs (*Canis familiaris*) are animals that were first domesticated by human for many purposes. In North Sulawesi Province, especially in Minahasa, dog meat has become an alternative protein source like chicken meat, pork, beef, etc; it is popular as RW meat (Rintek Wu-uk). It has now become one of the most saleable foods in many Minahasan's restaurants and supermarkets in Manado city and other places. People get rabies from the bite of an animal with rabies (rabid animal). It is quite rare that people may get rabies if infectious material from a rabid animal such as saliva gets directly into their eyes, nose, mouth or a wound, but it is also possible that it could happen. Because rabies is a fatal disease, the aims of this study are: firstly to obtain information on the rabies status of dogs in the wet markets; secondly, to obtain information on the amount of dog-meat purchased in the wet market; and thirdly, to obtain information in order to establish the appropriate strategy in preventing and controlling rabies in North Sulawesi Province. Samples of dogs' heads were bought at several wet markets in Manado city, Airmadidi and Langowan. Samples were analyzed using the Seller diagnostic method and then continued with the FAT in the Animal Health laboratory, Manado. It was found that from a total of 103 dog-head samples obtained, 8 were positive rabies (7.8%). Subsequently, from 47 additional dog-head samples taken, 5 were positive rabies (10.6%). Furthermore, rabies cases in dogs at the Animal Health Laboratory from the period of 2000 - 2006 were between 68% - 85.6% with an average of 602 tests per year and the number of people bitten by dogs was 909 per year. In short, there is a serious threat of rabies in Manado, Airmadidi and Langowan wet markets to the butchers, sellers and consumers of RW meat. Perhaps most concerning was the fact that a number of dogs never showed any signs of rabies.*

**Keywords:** rabies, *Canis familiaris*, wet markets, FAT.

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## Introduction

Rabies is an infectious viral disease that affects the nerves system of humans and other mammals for example dogs, cats and monkies. Rabies is mainly caused by the Lyssa virus, *Rhabdoviridae* family, known as “penyakit anjing gila” (Indonesia), “Tollwut” (Germany), “La Rage” (French) or hydrophobia (fear of water) and it is a zoonotic disease.

From abroad, it was reported that more than 90% of all animal cases of rabies occurred in wildlife animals such as raccoons, bats, foxes and skunks and less than 10% occurred in domestic animals like cats, cattle and dogs as most often reported rabid (Centers for Disease Control, 2003).

In Indonesia, the cases of rabies is the other way round in that most rabies cases are reported in dogs, cats and monkies (Ressang, 1986). In North Sulawesi province, cases of rabies were firstly reported in 1957, occurring in the Sangihe islands (Livestock Services, 1996). The number of rabies cases has been increasing in pets (dogs being most often reported rabid) as well as in humans bitten by rabid animals (The Animal Health Laboratory-Manado).

Dogs (*Canis familiaris*) are animals that were first domesticated by humans for many purposes, for example hunting dogs, watch dogs, dogs for the deaf, guide dogs, news dogs, Search and Rescue (SAR) dogs, etc (Tanjung, 1992). In North Sulawesi Province, especially in Minahasa, dog meat has become an alternative protein source like chicken meat, pork, beef, etc; it is popular as RW meat (Rintek Wuuk). Based on the survey done by Tolu (1993), from a total of 60 respondents in Ratahan regency, 74% of their protein needs was from dog-meat with an average of 471.64 grams per month per person. Because the demand of dog-meat has been increasing, nowadays we can easily buy dog or dog-meat in several wet markets in Manado, Airmadidi and Langowan. The biggest dog-meat market in North Sulawesi Province is in Karombasan wet market in Manado. RW as food has now become one of the most saleable foods in many Minahasan restaurants and supermarkets in Manado city and other places in Minahasa.

People get rabies from the bite of an animal with rabies (rabid animal). It is quite rare that people get rabies if infectious material from a rabid animal, such as saliva, gets directly into their eyes, nose, mouth or a wound, but it is also possible that it could happen. Most of the human fatalities associated with rabies occur in people who fail to seek

medical assistance and who are unaware of their exposure. In Indonesia, rabies is terrified because of vaccine to prevent human rabies is expensive and most of the people worries of death from rabies.

### ***Aims***

Because rabies is a fatal disease, the aims of this study are:

- Firstly to obtain information on the rabies status of dogs in the wet markets;
- Secondly, to obtain information on the amount of dog-meat purchased in the wet market;
- Thirdly, to obtain information in order to establish the appropriate strategy in preventing and controlling rabies in North Sulawesi Province.

## **Materials & Methods**

### ***Materials***

A total of 103 dogs' heads samples were bought at several wet markets in Manado city (Paal Dua, Bersehati and Karombasan), Airmadidi and Langowan in 1994. A total of 47 dog-head samples were taken subsequently at the Karombasan wet market in 1995. All dog-head samples in the plastic bags were stored into cooled boxes and then sent to the Manado Animal Health laboratory.

### ***Seller diagnostic procedure***

One by one the dog-head samples were put on the surgery (fixation) board, and one by one of dog-heads were opened in order to take the hippocampus (Amon's horns) out. Touched specimens of Amon's Horn were prepared and seller colors were put on it. After 30 seconds the touched-specimens were washed under tap water and let to dry at room temperature. All the touched-specimens were then observed under a microscope with 40-100 times enlargements, to see if there were any of Negri Bodies.

### ***Fluorescent Antibody Test (FAT) procedure***

The touched-specimens of Amon's Horns samples that had been tested above then were redone, this time in an active 4°C fixation. After 4 hours, anti-rabies fluorescent conjugated droplets were put on it and all

the samples were placed into the incubator at a temperature of 37°C. Thirty minutes later, all the touched-specimens were taken out from the incubator and then all of those touched-specimens were washed using PBS with pH of 7.2 – 7.4. The areas where antigen is present can be visualized as fluorescent-apple green areas using a fluorescent microscope. If there is no any rabies virus, there will be no staining.

## Results & Discussions

By using the Seller diagnostic procedure all the samples were found negative of Negri bodies. However, by using the FAT procedure from a total of 103 dog-head samples obtained, 8 samples were found positive rabies (7.8%) and subsequently, from a total of 47 dog-head samples, 5 samples were found positive rabies (10.6%). Furthermore, rabies cases in dogs at the Animal Health Laboratory from the period of 2000 - 2006 were between 68% - 85.6% with an average of 602 tests per year and the number of people bitten by dogs was 909 per year (Table 1). The anamneses test proved that most of the dogs that were positive rabies never showed any signs of rabies before they were slaughtered. So that, pet owners do not realize their pet (mainly dog) get rabies. The increasing number of people bitten by dogs and the number of rabid dogs indicated that the control and prevention of rabies is needed immediately.

Table 1. The number of People bitten by dogs and the number of dogs brought to the Animal Health Laboratory for having rabies test between the years 2000 – 2006.

Year	People bitten by dogs	Dogs Bite	Positive	Percent
2000	512	364	250	68,7
2001	603	443	322	74,4
2002	928	472	392	83,1
2003	1164	732	577	78,8
2004	1095	794	636	80,1
2005	1017	688	528	76,7
2006	1045	720	616	85,6
Sum	6364	4213		
Average	909.14	601.86		

Source: The Animal Health Laboratory, Manado.

An interesting piece of information we got from the butchers in the Karombasan wet market, was that the number of dogs sold from

Monday to Friday was about 5 – 15 dogs, and it increased on Saturday or Sunday, between 100 – 125 dogs. Dog prices vary between Rp.175, 000 – Rp.300, 000; depend on the size or weight of the dog. Dog-meat price is Rp. 21,000 per kg. So, it can be said that the prospect of dog-meat is as good as pork and chicken meat. However, as we have remarked above, not only must we consider the economic effects of the particular market but we must consider the rabies effects upon the public health and safety.

## **Conclusion**

In short, there is a serious threat of rabies in the Manado, Airmadidi and Langowan wet markets to the butchers, sellers and consumers of RW meat. Perhaps most concerning was the fact that a number of dogs never showed any signs of rabies.

## **References**

Centers for disease Control & Prevention. 2003.  
<http://www.cdc.gov/ncidod/dvrd/rabies>.

Dinas Peternakan Prop. Sulawesi Utara (Livestock Services of North Sulawesi Province), 1996. Laporan Pemberantasan Rabies di Propinsi Sulawesi Utara

Ressang, AA. 1986. Penyakit Viral Pada Hewan. UI-Press. Jakarta.

Tanjung, S. 1993. Training Manual K-9 Foundation. Surabaya

Tolu, SSL. 1993. Pengaruh Pendapatan dan Selera Masyarakat Terhadap Konsumsi Daging Anjing Di Kecamatan Ratahan Kabupaten Minahasa. Skripsi Fakultas Peternakan Universitas Sam Ratulangi. Manado.

# Estimation of Relative Efficiency of Indirect Selection for Weaning Weight Base on Birth Weight Using Maternal Effect Model on Landrace Cross Breed Pigs

Sri Bandiati Komar Prajoga<sup>2</sup>

## Abstract

*This research was conducted at the laboratory of Cooperative of Indonesian Pigs Holder, Cisarua, Lembang on September 2005. The objectives of this study were to estimate response to selection, correlated response to selection and relative efficiency of indirect selection. The data comprised 329 birth weight records and 329 weaning weight records of progeny from 20 sires and 40 dams of Landrace Cross Breed pigs. Variance Component, heritability and genetic correlation were estimated by Restricted Maximum Likelihood (REML) using Maternal Effect Model with the program of VCE 4.2. Best Linier Unbiased Prediction (BLUP) estimated Breeding Values of birth weight and weaning weight. Fixed effect was sex, parity of dams and litter size. Heritability for birth weight and weaning weight were  $0.314 \pm 0.035$  and  $0.316 \pm 0.035$ , as high category. The genetic correlation between birth weight and weaning weight was  $0.99 \pm 0.007$ . The accuracy of selection of birth weight and weaning weight were 0.562 and 0.560. Response to selection of birth weight was 0.48 kg and Response to selection of weaning weight was 0.484 kg. While selection intensity was 23.13% for 90 sows and 1.79% for 6 boars (sex ratio 1 ♂: 15 ♀). Either for correlated response to selection was 0.478 kg for the same selection intensity. The relative efficiency of indirect selection for weaning weight base on birth weight was 0.995. The result shows that selection of weaning weight could be carried out based on birth weight, if the economic aspect was included in the selection program. The weaning character may be economically better for an early measured correlated character, such as birth weight.*

**Key word:** Response Selection, Correlated Respond to Selection, Efficiency Relative of Indirect Selection, Landrace Cross Breed Pigs.

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## Introduction

The Landrace Cross Breed belongs to family *Suidae*, Genus *Sus*, Species *Sus scrofa*. They have several domesticated wild population (*sus scrofa*). Landrace is new breed in the United States. The first Landrace hogs were imported from Denmark to United States in 1934.

The origin country is Denmark, they imported also to Indonesia and distributed to around Java Island. Landrace has white skin color and length bodies. The American Landrace Association, Inc. was formed in 1950. In the disqualification of the breed are listed: crooked short, broad, turned-up snouts, upright ears, black hair, short bodies, very deep sides, underdeveloped udder, with less than 12 teats, very sickled hocks, very broad back and small hams

The laboratory of Breeding Centre and Development of cooperative of Indonesian pig holder were established as non Government Institution to cultivate local pig and crossed to imported boars for developing Cross Breed Population. As problem solving, the cooperative of Indonesian Pig Holder tried to select the available local pigs to produce elite sows for increasing the genetic quality. It is not easy, especially money and time problems. However, crossing to imported proven boars for building New Cross Breed animal to increased the productivity and adaptation ability for tropics climate and diseases.

Selection usually determined as an activity of keeping the good pigs to breed, and culling the pigs with low quality. In quantitative genetic, selection is an effort to increase the frequency of gene characteristic that we expect. To do the selection, there are some needed genetic parameters, which are heritability ( $h^2$ ), breeding value (BV), and genetic correlation ( $r_g$ ). These genetic parameters can be estimated if the data of recording for all pigs are available. For these reason, it may be of advantage to record daily gain during growth. Correlation between two characters can be used for selection. Genetic correlation between birth weight (BW) and weaning weight (WW) was positive high (Warwick, et al.1995).

The productivity and reproduction are quantitative traits as expression of combination between genetic factor and environment. The average of BW of Cross Breed Swine was 1.238 kg and average of WW was 15,05 kg (Widodo dan Hakim, 1979).

Genetic parameter can be estimated by using half sib correlation with nested experimental design, full sib and offspring-

parent regression (*Falconer and Mackay*, 1995). In year, 2001 *Anang* et al. have developed computer program for Cumulative Model (CM), Multiple Trait Model (MTM), Fixed Regression Model (FRM) and Random Regression Model (RRM) using statistical analysis animal model for REML to estimate genetic parameter. They needed the pedigree information without to design experiment before.

Correlation between two traits is of interest for three reasons. *Firstly*, in connection with genetic reason of correlation through pleitropic action gen. *Secondly*, in connection with the changes brought about by selection and *third*, in connection with natural selection.

Correlation between two traits causes a correlated respond to selection. If selection is applied to, one trait, other trait changes.

Correlation is not only between two traits, but it can be also between repeated measurements in different period of production.

Besides the value of heritability ( $h^2$ ) and genetic correlation ( $r_g$ ) that give the influences to selection process, the intensity of selection also becomes very important to reach the genetic progress. Intensity of selection ( $z/p$ ) is the index, which is got by dividing height of the ordinate at point of truncation ( $z$ ) with the swine proportion, which is selected ( $p$ ); this value can be seen on the table (*Falconer and Mackay*, 1996). Intensity of selection in animal breeding should have sire and dam candidates, so the total intensity of selection, which is combine intensity between boars and sows, will be got.

In the same level of intensity of selection, indirect selection will be estimated which one is more effective, throughout the checking: if the result of multiplication of BW selection accuracy ( $\sqrt{h_B^2}$ ) with genetic correlation between BW and WW ( $r_{gBw}$ ) is bigger than the accuracy of WW ( $\sqrt{h_W^2}$ ), so that the selection will be better for indirect selection and the result will be more than 1 (one) (*Falconer and Mackay*, 1996).

The objectives of this study were to estimate response to selection, correlated response to selection and relative efficiency of indirect selection.

## Material and Method

The object of this research is 329 Landrace Cross Breed as progeny from 20 sires and 40 dams, which recorded from 2005 the laboratory of Cooperative of Indonesian Pigs Holder. The data (329 records of BW and 370 records of WW) taken from this research is used as material. The research method is analytic descriptive.

The estimation of variance component and correlation genetic used Animal Model for REML, and the program used Variance Component Estimation – VCE 4.2 (*Groeneveld*, 1998) Breeding value is estimated by Multiple Trait Model (MTM) Best Linier Unbiased Prediction (BLUP) using Maternal Effect Model for PEST programs. Fixed effect was sex, parity of dams and year season.

### ***The statistic formula for Animal Model:***

$$Y_1 = X_1 B_1 + Z_1 U_1 + W_{m1} + e_1 \quad \text{and} \quad Y_2 = X_2 B_2 + Z_2 U_2 + W_{m2} + e_2$$

Where:

$Y_1$  and  $Y_2$  = vector of observations of BW and WW

$X_1$  and  $X_2$  = design matrix connecting the observations to fixed effects of BW and WW

$Z_1$  and  $Z_2$  = design matrix connecting the observations to random effects of BW and WW

$W_1$  and  $W_2$  = design matrix connecting the observations to Maternal effects of BW and

WW

$b_1$  and  $b_2$  = vector of fixed effects of BW and WW

$u_1$  and  $u_2$  = vector of random effects of BW and WW

$m_1$  and  $m_2$  = vector maternal effect of BW and WW

$e_1$  and  $e_2$  = vector residuals of BW and WW

With Mixed Model Equations:

$$\begin{bmatrix} Y_1 \\ Y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \begin{bmatrix} m_1 \\ m_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

$$Var(u) = G = \begin{bmatrix} \text{cov}(u_1, u_1) & \text{cov}(u_1, u_2) \\ \text{cov}(u_2, u_1) & \text{cov}(u_2, u_2) \end{bmatrix} = \begin{bmatrix} A\sigma_{g11}^2 & A\sigma_{g12}^2 \\ A\sigma_{g21}^2 & A\sigma_{g22}^2 \end{bmatrix} = G \otimes A$$

$$Var(e) = R = \begin{bmatrix} \text{cov}(e_1, e_1) & \text{cov}(e_1, e_2) \\ \text{cov}(e_2, e_1) & \text{cov}(e_2, e_2) \end{bmatrix} = \begin{bmatrix} I\sigma_{e11}^2 & I\sigma_{e12}^2 \\ I\sigma_{e21}^2 & I\sigma_{e22}^2 \end{bmatrix} = I \otimes R$$

$$h_1^2 = \frac{\text{cov}(u_1, u_1)}{\text{cov}(u_1, u_1) + \text{cov}(e_1, e_1)}$$

$$h_2^2 = \frac{\text{cov}(u_2, u_2)}{\text{cov}(u_2, u_2) + \text{cov}(e_2, e_2)}$$

$$\text{Genetic correlation (r}_g\text{)} = \frac{\text{cov}(u_1, u_2)}{\sqrt{\text{cov}(u_1, u_1) + \text{cov}(u_2, u_2)}}$$

$$\text{Phenotypic correlation (r}_p\text{)} = \frac{\text{cov}(u_1, u_2) + \text{cov}(e_1, e_2)}{\sqrt{\{\text{cov}(u_1, u_1) + \text{cov}(e_1, e_1)\}\{\text{cov}(u_2, u_2) + \text{cov}(e_2, e_2)\}}}$$

Where:

$$\sigma_{g1}^2 = \text{cov}(u_1, u_1) \quad = \text{Genetic variance for BW}$$

$$\sigma_{g2}^2 = \text{cov}(u_2, u_2) \quad = \text{Genetic variance for WW}$$

$$\sigma_{e1}^2 = \text{cov}(e_1, e_1) \quad = \text{Phenotypic variance for BW}$$

$$\sigma_{e2}^2 = \text{cov}(e_2, e_2) \quad = \text{Phenotypic variance for WW}$$

$$h_1^2 \quad = \text{Heritability for BW}$$

$$h_2^2 \quad = \text{Heritability for WW}$$

$$r_g \quad = \text{Genetic correlation}$$

$$r_p \quad = \text{Phenotypic correlation}$$

A	= Matrix for relationship
I	= Matrix for identity

The estimation of intensity of selection by dividing the height of phenotype normal curve ordinate with the proportion of selected swine; it can be seen on the table. Intensity of selection of selection ( $i = z/p$ , where  $i$  = intensity of selection,  $z$  = height of the ordinate at point of truncation,  $p$  = proportion of selected swine, *Falconer and Mackay, 1996*).

The Response to selection for WW was estimated by multiplying the total intensity of selection with heritability of WW and phenotype standard deviation WW ( $R_1 = i h_1^2 \sigma_p$ , where:  $R_1$  = response to selection,  $h_1^2$  = heritability of BW,  $i$  = total intensity of selection  $[(i \text{ boars} + i \text{ sows})/2]$ ,  $\sigma_p$  = phenotype standard deviation of BW).

The Response to selection for WW was estimated by multiplying the total intensity of selection with heritability of YW and standard deviation ( $R_2 = i h_2^2 \sigma_p$ , where:  $R_2$  = response to selection,  $h_2^2$  = heritability of WW,  $i$  = total intensity of selection  $[(i \text{ boars} + i \text{ sows})/2]$ ,  $\sigma_p$  = phenotype standard deviation of WW).

Correlated response to selection between BW and WW can be estimated by multiplying total intensity of selection with the accuracy of selection for BW, accuracy of Selection for WW, genetic correlation between BW and WW and Phenotype standard deviation of WW ( $CR_2 = i h_1 h_2 r_{g12} \sigma_{p2}$ , where  $CR_2$  = response to selection of BW and WW,  $i$  = total intensity of selection  $[(i \text{ boars} + i \text{ sows})/2]$ ,  $h_1$  = accuracy of individual selection for BW records ( $\sqrt{h_1^2}$ );  $h_2$  = accuracy of individual selection for WW ( $\sqrt{h_2^2}$ );  $r_{g12}$  = genetic correlation between BW and WW,  $\sigma_{p2}$  = phenotype standard deviation of WW (*Falconer and Mackay, 1996*).

Relative efficiency of indirect selection is estimated by dividing correlated response to selection with response to selection of WW, or the result of dividing between intensity of selection multiplies with accuracy of selection of BW multiplies and intensity of selection

with accuracy of selection of WW, then multiplied with genetic correlation between them both (*Falconer and Mackay, 1996*).

$$Q = \frac{CR_2}{R_2} = \frac{ih_1}{ih_2} r_{g12}$$

## Result and Discussion

BW and WW in the laboratory of Cooperative of Indonesian Pigs Holder i is recorded every 0 and 56 days after partus. The data comprised 329 BW records and 329 WW records of progeny from 20 sires and 40 dams. Recording started from body weight of zero day to mature weight. After all data get tabulated and given code according to REML, then it can be provided in first data description structure as a result from Excel, as written on table 1:

Table 1. Structure Data of BW and WW records in the laboratory of Cooperative of Indonesian Pigs Holder.

Trait	n	Female				Male				
		Average (kg)	min. (kg)	Max. (kg)	CV (%)	n	Average (kg)	min. (kg)	Max. (kg)	CV (%)
BW	175	1.949 ±0.263	0.995	2.31	17.63	154	1.503 ±0.260	1.019	2.098	17.326
WW	175	13.563 ±2.356	9.500	20.00	19.38	154	13.92 ±2.353	9.00	19.00	17.47

Where: CV= Coefficient Variation; n = total data.

On Table 1 above the average of BW of female was 1.949 ±0.263 kg and Coefficient Variation was 17.63%, when the range of data between minimum value to maximum value were 0.995 kg to 2.31 kg. The average BW of male was lower than female (1.503 ±0.260 kg) and the Coefficient Variation was 17.326%, when the range between minimum value to maximum value were 1.019 kg to 2.098 kg. Base on both of Coefficient Variation The conditions of data were heterogenic, and will be effective for doing selection of BW.

The average of WW of female was 13.563 ±2.356 kg and Coefficient Variation was 19.38%, when the range of data between minimum value to maximum value were 9.50 kg to 20.00 kg. The average WW of male was higher than female (13.92 ±2.353 kg) and the Coefficient Variation was 17.47%, when the range between minimum value to maximum value were 9.00 kg to 19.00 kg.

The structure data shows, that BW was higher and WW was lower in compare to the result of several references. In Year 1979 *Widodo and Hakim* has published that male and female BW and WW of local breed were 1.238 kg and 15.05 kg. There were deference result causes of management and year season.

For the better data should't have the big deviation standard, so the selection process won't loose so much swine. But, to select a group of swine with the big Coefficient Variation-CV of data will give the effective result, if only not less than 10% or closer to 0, because the genetics progress will be very small and intend to reach the selection plateau.

Tabel 2 shows that the analysis result from BW records shows Lower value of heritability ( $0.314 \pm 0.035$ ) in compare to the value of heritability of WW ( $0.316 \pm 0.035$ ), according to the selection purpose, not to waste so many production cost. So that can be pointed wich BW is the most efficient to do the selection. In a line with *Falconer and Mackay* (1996), that to wait the selection process the correlated recording character, it needs big budget for measurement then selection has to be done with correlated indication.

Table 2. Heritability, Accuracy of Selection, Standard Deviation and Genetic Correlation

Trait	Heritability ( $h^2$ ) $\pm$ se	Accuracy ( $\sqrt{h^2}$ )	Maternal efect ( $m^2$ ) $\pm$ se	$r_g$ (WB-WW)
BW	$0.314 \pm 0.035$	0.562	$0.372 \pm 0.033$	$0.990 \pm 0.007$
WW	$0.316 \pm 0.035$	0.560	$0.368 \pm 0.033$	

The higher heritability value among BW and WW records is on WW ( $0.316 \pm 0.035$ ) included the high category, according to *Dalton's* (1981). If the selection is held, the high heritability will be better for the effectiveness of the process.

The decision will be made not only based on the heritability value, but also the genetics correlation between both the variables, if selection will be done indirectly. The heritability of the two process of recordings and genetics correlation should be known. The point is agreed by *Falconer and Mackay* (1996) who come to public with the experiment called *double selection experiment*.

Table 2 also shows that the value of selection accuracy will follow the value of heritability from BW (0.562) and WW (0.560), because the selection accuracy is the base from heritability value ( $\sqrt{h^2}$ ).

This value is needed in counting the correlated response and the indirect selection relative efficiency for WW based on BW records.

The genetics correlated value between BW and WW records can be seen on Table 2. This genetic correlation is needed to select the swine indirectly. The value shows the tightness relationship between this two kinds of recording that get the influence by the breeding value. The genetics progress in BW will give the progress of genetics to the WW. The genetics correlation value of BW and WW provides the highest value ( $0.990 \pm 0.007$ ), including the high positive category.

Breeding value is the value from swine genetics quality for a certain character that is given relatively based on the individual in population. The estimation of this value is the main factor to evaluate the excellent swine genetics because a half of the value will be given to the next generation. The research result shows that the range of positive breeding value is between 0.012 kg to 1.687 kg for boars and between 0.017 kg to 1.337 kg for sows more than the average of population production. For the lowest is between -0.008 to -0.997 kg for boars and -0.004 to -1.149 kg for sows under the average of population. In BLUP for PEST program, this value has the position of positive and negative and if zero (0) is equivalent the average.

Based on the analysis there were 90 sows (23.13%) that were more than the average and selected as the dams. There are 6 heads (1.54%) of selected boars based on their breeding value that were over the population average. Obviously, the genetics potency for growth character for boars is higher than the sows. According to this ranking, will be selected some swine that will get involved in the process, to gain the intensity of selection.

The intensity of selection is the average of deviation for all selected swine in one phenotypic standard deviation unit. In simply, intensity of selection is excess average of selected swine in the population average. It depends on number of selected swine. If selection involves big number of swine, it will make intensity of selection and genetic progress run slowly. It refers to *Kinghorn* (1992) that the intensity of selection inversely proportionate with the number of the selected swine.

The Intensity of selection used in this program is 1.343 from 23.13% (90 sows) and 2.411 from 1.79% (7 boars). The average intensity of selection between dams and sires is 1.877. The optimum intensity of selection is 1.931, while proportion of selected swine was 23.13% for sows (90 heads) and 1.5% for boars (6 heads). The sex ratio between boars and sows will be 1 (one) to 15 (fifteen).

The Response to selection for BW was estimated by multiplying the total intensity of selection with heritability of BW and standard deviation of BW ( $R_1 = i h_1^2 \sigma_p$ , where:  $R_1$  = response to selection of BW,  $h_1^2$  = heritability of BW,  $i$  = total intensity of selection  $[(i \text{ boars} + i \text{ sows})/2]$ ,  $\sigma_p$  = phenotype standard deviation of BW).

Table 3. Estimation of Respond to Selection for BW Records in Difference Intensity of Selection

Selected Boars (%)	n (head)	Selected sows (%)				
		3,85	7,71	15,42	19,28	23,13
		15	45	60	75	90
0,77	3	0,612	0,552	0,534	0,519	0,510
1,02	4	0,600	0,540	0,522	0,507	0,498
1,28	5	0,591	0,530	0,512	0,498	0,488
1,54	6	0,583	0,523	0,505	0,490	0,480
1,79	7	0,569	0,509	0,491	0,476	0,467

Table 3 shows that the proportion of selected swine affects the response to selection. The more proportion of selected swine will decrease intensity of selection, the more response to selection will decrease as well. The highest response (0.612 kg) is reached at 3,85% (15 heads) selected sows and 0,77% (3 heads) selected boars, in ratio 3 boars to 15 sows. However, it is recommended that the ratio for swine is 6 (six) boars to 90 sows to avoid inbreeding, which has response to selection 0.480 kg in range, and sex ratio 1 ♂: 15 ♀.

The Response to selection for WW was estimated by multiplying the total intensity of selection with heritability of WW and standard deviation of WW ( $R_2 = i h_2^2 \sigma_p$ , where:  $R_2$  = response to selection for weaning weigh,  $h_2^2$  = heritability of WW,  $i$  = total intensity of selection  $[(i \text{ boars} + i \text{ sows})/\text{two}]$ ,  $\sigma_p$  = phenotype standard deviation of WW).

Table 4. Estimation of Respond to Selection for WW Records in Difference Intensity of Selection.

Selected boars (%)	n (head)	Selected sows (%)				
		3,85	7,71	15,42	19,28	23,13
0.77	3	0,618	0,557	0,539	0,524	0,514
1.02	4	0,606	0,545	0,527	0,512	0,502
1.28	5	0,596	0,535	0,517	0,502	0,492
1.54	6	0,588	0,527	0,509	0,494	0,484
1.79	7	0,575	0,514	0,496	0,481	0,471

Table 4 shows that the proportion of selected swine affects the response to selection. The highest response (0.618 kg) is reached at 3,85% (15 heads) selected sows and 0,77% (3 heads) selected boars, in ratio 3 boars to 15 sows. However, it is recommended that the ratio for swine is 6 (six) boars: 90 sows to avoid inbreeding, which has response to selection 0.484 kg in range, and sex ratio 1 ♂ : 15 ♀.

All swine involved on the table above have a higher Breeding Value over the population's average. However, it is different in the real practicing. In a group of swine is not allowed to involve more males, so only 3 – 5 boars which have the highest breeding value among them will be selected to mate 90 sows by using AI (Artificial Insemination) and will be replaced after 2 years, so the genetic progress that will be reached on next generation is in between 0.514 kg - 0.492 kg.

Response to selection will be showed on the next generation, and it will take time so long to wait until WW (56 days) ahead, therefore; we can predict it by estimating the correlation response between WW (56 days) and BW (0 days). It needs to be considered for saving budget to do the direct selection because of feeding a few non-productive swine.

The estimation of correlated response between WW (56 days) and is BW (0 days) a result of multiplying the total intensity of selection, accuracy of selection of BW, accuracy of selection of WW, genetic correlation between both of traits and standard deviation.

The objective of Selection program based on correlation trait is to enhance the selection accuracy. Based on that genetic parameter for those models, it is possible to predict if response to selection based on first indicator, (BW) can estimate response to selection of WW.

Table 5 below shows correlated response in different intensity of selection. Correlated response to selection between BW and WW can be estimated by multiplying total intensity of selection with the accuracy of selection for BW, accuracy of Selection for WW records,

genetic correlation between BW and WW and Phenotype standard deviation for WW ( $CR_2 = i h_1 h_2 r_{g12} \sigma_{p2}$ , where  $CR_2$  = response to selection of BW and WW,  $i$  = total intensity of selection  $[(i \text{ boars} + i \text{ sows})/2]$ ,  $h_1$  = accuracy of individual selection for BW records ( $\sqrt{h_1^2}$ );  $h_2$  = accuracy of individual selection for WW ( $\sqrt{h_2^2}$ );  $r_{g12}$  = genetic correlation between BW and WW,  $\sigma_{p2}$  = phenotype standard deviation of WW (Falconer and Mackay, 1996).

Table 5. Estimation of Correlated Respond to Selection for WW records. In addition, BW Records in the Difference Intensity of Selection.

		Selected sows (%)					
		(%)	3,85	7,71	15,42	19,28	23,13
Selected boars (%)	n (head)	15	45	60	75	90	
	0.77	3	0,609	0,549	0,531	0,517	0,507
	1.02	4	0,597	0,537	0,519	0,504	0,495
	1.28	5	0,588	0,528	0,510	0,495	0,485
	1.54	6	0,580	0,520	0,502	0,487	0,478
	1.79	7	0,567	0,507	0,489	0,474	0,464

The Table 5 above shows that the highest value of correlated response is 0.716 kg on proportion 3,85% (15 sows) and 0,77% (3 boars). Correlated response with highest intensity gives highest response than direct selection on WW (Table 4). If we refers to recommended ratio for swine as can be seen on Table 5, which is sex ratio 1 ♂ : 15 ♀, the similar proportion we can use is 1.54% (6 boars) and 23,3% (90 sows), which has correlated response 0.561 kg.

On the Table 5, proportion 23,3% (90 sows) and 1,79% (7 boars) is a prediction for genetic progress for the offspring with the lowest value of correlated response 0.546 kg. Genetic progress on BW will occur on WW, better than direct selection (Table 4), also if the economic aspect was included in the selection program, the weaning weigh character may be economically better for an early measured correlated character, such as BW.

The research result shows that the main key to run indirect selection program is the value of heritability of BW must be higher than heritability of WW. Therefore; the accuracy for BW will be higher than WW and the expected procedure will be reached by maximizing the variation of selected swine with the average of other swine and

maximizing the heritability by decreasing the environment variance as well.

Relative efficiency of indirect selection is estimated whether direct selection WW is not better than indirect selection based on BW to select growth trait. Indirect selection relative efficiency got by dividing correlated response with response to selection of WW, and simplified by dividing the heritability of BW with the heritability of WW and multiplied with genetic correlation between BW with WW.

Relative efficiency of indirect selection was 0.995. It means indirect selection was almost better than direct selection. The value was big enough if we compares with quantity aspect, but if we observe further that earlier selection would save time and could minimize the interval of generation, and also will save money because we do not have to feed unproductive swine.

The value of selection accuracy and genetic correlation indicates the accuracy level of procedure of selection, that is:

1. Maximizing phenotypic variance of selected swine with other phenotypic average in population,
2. Maximizing heritability by decreasing environment variance. In conclusion, indirect selection will be done if only it is better than direct selection.

## **Conclusion and Suggestion**

The highest response to selection of BW (0.612 kg) and WW (0.618 kg) is reached at 3.85% (15 heads) selected sows and 0,77% (3 heads) selected boars, in ratio 3 boars: 15 sows. However, it is recommended that the ratio for swine is 6 boars: 90 sows (sex ratio 1 ♂: 15 ♀) to avoid inbreeding. In this research, the similar ratio value, which has response to selection 0.480 kg for BW and 0,484 kg for WW. At the same intensity, the correlated response between BW with WW is 0.478 kg. The relative efficiency value of indirect selection for YW according to WW is 0.995.

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## References

Anderson, A.L. 1957. Swine Management including Feeding and Breeding, Second Edition, J.B. Lippincott Company. Chicago, Philadelphia, New York.

Anang, A. 2002. Pendugaan Nilai Pemuliaan dengan Best Linier Unbased Prediction (BLUP). Fakultas Peternakan. Universitas Padjadjaran. Bandung 3; 13 – 17.

Cameron, N.D. 1997. Selection Indices and Prediction of Genetic Merit in Animal Breeding. CAB International. Wallingford, United Kingdom. 38-39.

Dalton, D.C. 1981 an Introduction to Practical Animal Breeding. Granada Publishing Limited. London.

Falconer D.S and Mackay, T.F.C. 1996. Introduction to Quantitative Genetic. (Fourth edition) Longman Malaysia. . 209 – 246.

Groeneveld, E. 1998. VCE4.2 User's Manual. Institute of Animal Husbandry and Animal Behavior. Federal Agriculture Research Centre. Germany.

Kinghorn, B. 1992. Principles of Genetic Progress in Animal Breeding, Animal Breeding the Modern Approach. Post Graduate Foundation in Veterinary Science. University of Sidney. Australia.

Legates, J.E. dan Warwick, E.J. 1990. Breeding and Improvement of Farm Animal (eighth edition) Mc. Craw-Hill Publishing Company. Singapore. Hal. 140-142.

Lynch, M. and B. Walsch. 1999. Genetic and Analyses of Quantitative Traits. Sanauer Assocites Inc.

Mielenz dan Schüler. 1999. Zuchtwertschätzung. Lehrmaterialien zur Nutztiergenetik. Institut für Tierzucht und Tierhaltung mit Tierklinik, MLU-Halle Wittenberg.

Warwick, E.J., Maria Astuti, Hardjosubroto. 1995. Pemuliaan ternak. Gadjah Mada University Press. Yogyakarta.

Widodo, W. dan L. Hakim. 1976. Pemuliaan ternak. Universitas Brawijaya Malang.

## Clove Oil (*Eugenia aromatica*) and Potassium Hydroxide (KOH) as A Semi Permanent Stain on Nematodes and Acanthocephalan Worms of Marine Fishes

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### Abstract

*The study on the using of 10% Potassium Hydroxide (KOH) and clove oil (*Eugenia aromatica*) for semi-permanent staining of aquatic parasitic worms was performed. Thick outer-lining cuticle of nematodes and acanthocephalan worms often prevents conventional staining process. 10% Potassium Hydroxide (KOH) and clove oil (*Eugenia aromatica*) are staining substances that can potentially be utilized to make semi permanent stain on nematodes and acanthocephalan worms. Determination of staining effectivity is calculated as percentage of identifiable organs per slide compared to conventional method of standard acetocarmine staining (positive control) on trematode worms. Results show that 10% KOH and the clove oil staining on nematodes and acanthocephalan worms are as effective as the standard acetocarmine staining on trematode worms. We suggest that these both substances could be use as a semi permanent stain.*

*Key word:* Clove oil (*Eugenia aromatica*), potassium hydroxide, semi permanent stain, nematodes, acanthocephalan.

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## Introduction

Parasites are organism live in the outer or within the host body. Parasites were divided into ectoparasites and endoparasites. One of the endoparasites is worm parasite that could affect all animals including aquatic animals. Worm is categorized as parasites which live within the hosts' body. The negative impacts of the parasitic worm infection in aquatic animals are including of inhibitions in growth, body weight and reproduction. Anti-worm medication (anthelmintic) is commonly used to eliminate these parasites, but unfortunately most of the anthelmintic drug is effective only to one kinds of worm, therefore, to ensure effective drug administration accurate parasite identification is required (Sukarban 1995). This can be achieved by identifying the types of parasitic worm using the staining techniques. One of the staining dyes that give good result is acetocarmine. This dye is usually use for trematodes and cestodes, and not for nematodes and acanthocephalan due to the thick outer-layer of cuticle. The dye characteristic use for both worms (for nematodes and acanthocephalan) is semi-permanent.

According to Lawrence (1991), the most semi-permanent dye staining using for identification of nematodes is combination between 70% alcohol and glycerin. For identification of acanthocephalan, mounting technique is the most common use because of identification usually only based on the number of hook in their proboscis (Properna 1996). Commonly, the staining for nematodes and acanthocephalan was not properly enough due to the cuticle structure and the thick of integument prevent the penetration of the dye to the worm tissue.

The present study deal with the use of 10% KOH and clove oil for staining of the nematodes and acanthocephalan worms in order to find out the ideal substances for nematodes and acanthocephalan staining that can suitable for worms identification.

## Materials and Methods

### **Worms Samples**

Nematodes, acanthocephalan and trematodes from marine fishes of Yellow tail, Grouper and Tuna were collected from area of Banten, Bali and East Nusa Tenggara on June 2005 to May 2006.

### **Staining Techniques**

Acetocarmine dye was use for permanent staining of trematodes.

1. Worm specimen was dipped on acetocarmine dye for 5-7 minutes until the worm was red colored.
2. Specimens were then dipped on 70% alcohol that contained 2-45 drops of Hydrochloric acid (HCl) to wipe of the over-stained color.
3. Dehydration step using a series of alcohols (70%, 85%, 95% and absolute).
4. The clearing step was done using a Xylol solution.
5. The final step was mounting of the specimen using a Canada balsam.

Staining procedure of the worms using 10% KOH and clove oil.

1. The worm specimens were dipped on 10% KOH for 30 – 60 seconds.
2. The specimens were dipped on clove oil for 15-30 seconds until the brown color was appeared.
3. The colored specimen were then dipped in serial alcohols for dehydration :
  - a. 70% Alcohol 15-30 seconds
  - b. 85% Alcohol 15-30 seconds
  - c. 95% Alcohol 15-30 seconds
  - d. 100% Alcohol 15-30 seconds
4. The final step was mounting of the specimen using a Canada balsam.

### **Assay of the Staining Result**

Assay of the semi permanent staining on the nematodes and acanthocephalan by the clear image of the morphologies of the stained organs was done. From this staining result, comparison between the semi-permanent and standard permanent staining was made by visual observation using a standard microscope.

### **Results and Discussions**

50 stained samples of worms from marine fishes (Tuna, Gropuer and Yellow Tail) were divided into 3 groups i.e. 4 samples of trematodes, 31 samples of acanthocephalan and 15 samples of nematodes.

Basically, the standard acetocarmine staining gave a good result on the specimen of trematodes, this due to the thinness of integument layer on the body of the worm. This permanent staining gave around 85% - 100% on the worms organs stained. Acanthocephalan is the worm that morphologically (their body thickness) is similar to the family of nematodes

This semi-permanent staining (10% KOH and clove oil) made an identification of the worm target organs more easily. On the acanthocephalan (31 samples) this staining method gave a variety in the effectivity value on each target organs. For proboscis organ, 24 samples could be identified (77%), proboscis space (77%), sex organs (testis and uterus) (80.64%). For female sex organs of acanthocephalan, the identification was based on the presence of the eggs on their body. The failure value on the identification for acanthocephalan was 21.79%

The target organs identification of nematodes was digestive tract (mouth, oesophagus and intestines) and sex organs (testis, speculum and uterus). The percentage value of semi-permanent staining method on the target organs identification were 100% for mouth identification, 93% for intestines, while for sex organs the percentage were more lower i.e. 73% (10 samples for testis, 7 samples for speculum and 1 sample for uterus) therefore the failure value on the identification of nematodes was 8.5%.

Basically, the chemical characteristics of clove oil and 10% KOH supporting the staining process of marine fishes parasites. Kalium hydroxide is a strong base, water soluble, tissue litic or as a keratoliticum (Muller, 1983). The ability of KOH as keratoliticum could improve the lysis process of nematodes worm cuticle and the thick integument of acanthocephalan; this action increase the absorption of dye to the internal organs. Clove oil content of *eugenol* an uncolored or very mild yellowish oil and will become brown if contacted with air; this process called oxidation (Linggih dan Wibowo 1988). From this oxidation process, clove oil stained the worm through the permeability of the worm body wall. The color character of clove oil is unaffected by the dehydration process, this due to the character of clove oil that mild dissolve in water but strong dissolve in alcohol, ether and oil Ketaren 1985).

Result of this semi permanent staining had different success percentage. The explanation of staining result of 10% KOH and clove oil on the standard morphology of the worm on each worm groups is based on the general identification (Soulsby 1982; Noble dan Noble 1989; Kusumamihardja 1995; Grabda 1991; Properna 1996).

## Nematodes

According to Leed (1965), basic identification on nematodes is head shape (mouth) and the posterior. Digestive tract of nematodes is a simple tube which comprise of mouth single layer cells lining from capsula buccali (not always present) and to oesophagus (Noble&Noble 1989). In the staining result of KOH and clove oil, mouth, oesophagus and intestine were seen and could be clearly recognize as well as the different in size and shape of the genital organs (Figure 1). The male nematode had reproduction organs of testis and spiculum.

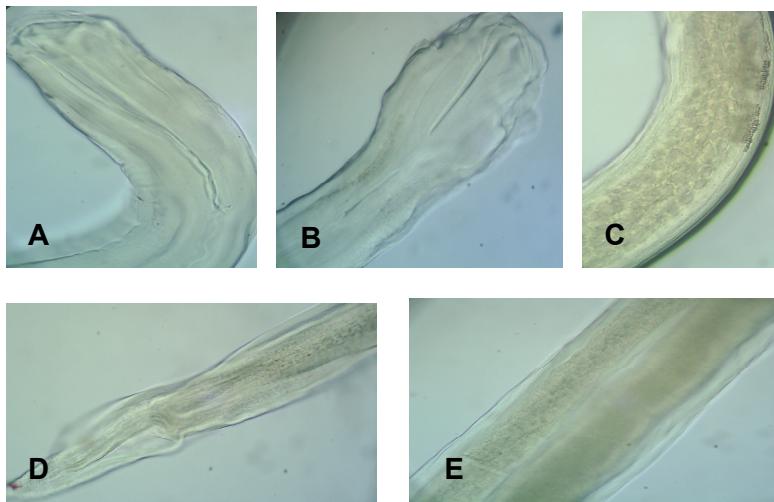


Figure 1. Staining result of the nematodes worm using 10% KOH and clove oil, there was a variation shape of the anterior region (A & B)(C); adult worm (C) and posterior region shape (D). The tubules bundle of male worm (E).

## Acanthocephala

Clove oil and 10 KOH used for this worms give a good result on the body shape image, i.e. the proboscis with the spike, neck, proboscis sack and genital organ. Acanthocephalan is the worm with cylindrical body shape and had a proboscis at the anterior (Grabda, 1991). Species identification of the acanthocephalan is based on the number of spikes in their proboscis (Properna, 1996). In this present study, acanthocephalan worm were stained clearly with their proboscis

(Figure 2). In the larvae stage and adult worm, there are no digestive and blood circulation tracts and the female worm is usually bigger than the male worm (Noble and Noble 1989).

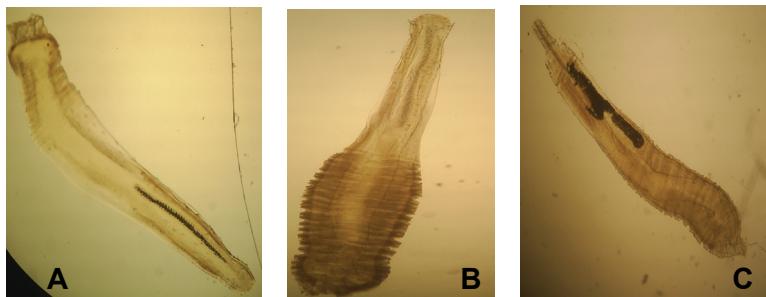


Figure 2. Staining result of the acanthocephalan worm with 10% KOH and clove oil. Proboscis position within the body (A) and worm morphology (B & C).

#### **The Average Effective Value of 10% KOH and Clove Oil Dyes.**

The average effective value of 10% KOH and clove oil indicated that the staining result is close similar to that of acetocarmine staining the standard dye for trematode worm. The number of divider is depend on the number of visible organs morphology and then justified with the standard identification of each worm groups.

$$1. \text{ Trematoda} : \frac{100 \% + 100 \% + 100 \% + 75 \% + 100 \% + 100 \%}{6}$$

$$= 95\%$$

$$2. \text{ Nematoda} : \frac{100 \% + 100 \% + 93 \% + 73 \%}{4}$$

$$= 91,5\%$$

$$3. \text{ Acanthocephala: } \frac{77 \% + 77 \% + 80,64 \%}{4}$$

$$= 78,21\%$$

Table 1. Average staining value of clove oil + 10%KOH and acetocarmine.

Worm Group	Acetocarmine (%)	Clove oil + 10% KOH
Trematode	95%	-
Nematode	-	91,5%
Acantocephala	-	78,21%

From the percentage value above, the significance interval of the staining result of 10% KOH and clove oil compared to acetocarmine could be determined using a statistical calculation as describe below.

$$P \pm Z\alpha/2 \sqrt{P(1-P)/n}$$

P = successful stained percentage

$Z\alpha/2$  = Z value two direction with level of significance 95%

n = Total sample number.

1. Trematoda =  $0,95 \pm Z\alpha/2 \sqrt{0,95(1-0,95)/4}$   
 $= 0,95 \pm Z(0,0250) \sqrt{0,95(0,05)/4}$   
 $= 0,95 \pm 0,05 \text{ (74% - 100%)}$
2. Nematoda =  $0,915 \pm Z\alpha/2 \sqrt{0,915(1-0,915)/31}$   
 $= 0,915 \pm Z(0,0425) \sqrt{0,915(0,085)/31}$   
 $= 0,915 \pm 0,08 \text{ (78% - 100%)}$
3. Acanthocephala =  $0,7821 \pm Z\alpha/2 \sqrt{0,7821(1-0,7821)/15}$   
 $= 0,7821 \pm Z(0,1089) \sqrt{0,7821(1-0,7821)/15}$   
 $= 0,7821 \pm 0,13 \text{ (70% - 86%)}$

Table 2. Interval value from staining result.

Worm Group	Interval
Trematode	74% - 100%
Nematode	78% - 100%
Acantocephala	70% - 86%

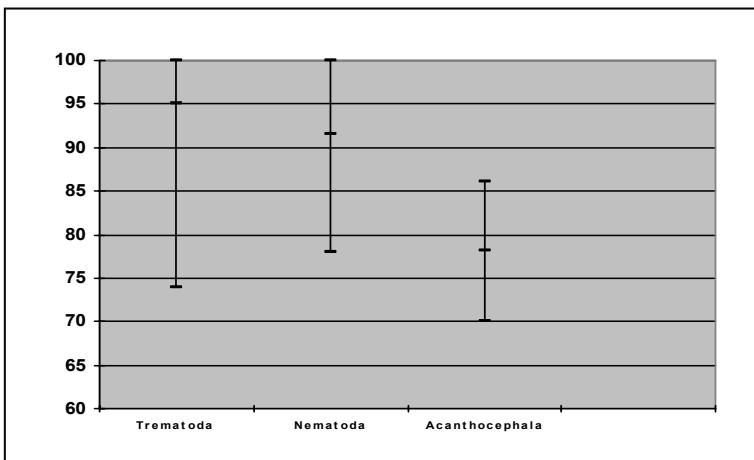


Figure 3. Percentage of Interval value of the staining result using 10% KOH and clove oil.

From the Table 2 and Figure 3, we can see that as a positive control acetocarmine had a significance interval value 74% - 100% on the staining of target organs (oral sucker, oesofagus, ventral sucker and genital organ). The percentage above were then compared to the significance interval value of semi permanent stain for nematode (78% - 100%), resulted that the interval value for nematode was within the interval value of trematode (acetocarmine). based on the result above, the significance interval value. In the acantocephlan, the percentage of significance interval value is 70%-86%, this value is smaller than trematode and nematode but in general the staining results is relatively good enough.

Staining using 10% KOH and clove oil give a good result on the coloring the digestive tract and the outer morphology especially anterior and posterior region of the worm. The disadvantage of the 10% KOH and clove oil is they could not be use for a long period only for about 5 – 6 months, this due to the character of this dye as a semi permanent staining and by time the dye will reducing its intensity as shown in Figure 4 with no internal organs visible within the worm body.

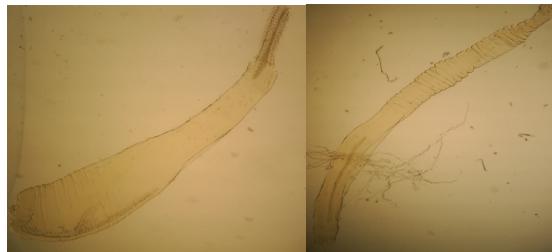


Figure 4. Acanthocephalan worm after 5 – 6 months staining period, the dye was reduce its intesity, the worm internal organs was not visible anymore.

### Conclusion

Based on all results mentioned above we concluded that 10% KOH and clove oil could be use as semi permanent staining dye for identification of acanthocephalan and nematode worms with limited period of 5 – 6 months.

### References

Grabda J. 1991. Marine Fish Parasitology. New York: Plenum Publishing Corporation.

Ketaren S. 1985. Pengantar Teknologi Minyak Atsiri. Jakarta : P N Balai Pustaka..

Kusumamihardja S. 1995. Parasit dan Parasitosis pada Hewan Ternak dan Hewan Piaraan di Indonesia. Bogor: Pusat Antar Universitas Bioteknologi IPB.

Lawrence, R. dan C. Thomas C. 1991. A Guide to Laboratory Procedures and Identification. Chicago: American Society of Clinical Pathologists Press.

Lee. 1965. The Physiology of Nematodes. London: Olner and Boy b Eiden Burgh.

Linggih, S. dan P Wibowo. 1988. Ringkasan kimia. Bandung : Ganesa Exact Bandung-ITB.

Muller, G.H., D.W. Scott. 1983. Small Animal Dermatology. Edisi ke-3. Philadelphia: WB Saunder Company.

Noble, E.R., G.A. Noble. 1989. *Parasitology: The Biology of Animal Parasites*. Edisi ke-5. Alih bahasa: Wardiarto. Yogyakarta: Gajah Mada University Press.

Radiopoetra. 1977. *Zoologi*. Jakarta : Erlangga.

Sukarban, S. 1995. *Farmakologi dan Terapi*. Edisi-4. Bagian Farmokologi Fakultas Kedokteran Hewan UI. Jakarta

Soulsby, E.J. L. 1982. *Helmints, Athropods and Protozoa of Domesticated Animals*. Edisi ke-7. London: Baillire Tindall.