REVITALISING THE LIVESTOCK INDUSTRY THROUGH TECHNOLOGY TRANSFER

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Editors:

H. Yaakub
E.A. Engku Azahan
H. Wahid
S. Ganabadi
S. Sukardi
S. Sumita
T.C. Loh
J.M. Panandam
Y. Jasmi
W.E. Wan Khadijah
Z.A. Jelan

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PREFACE

The Malaysian Society of Animal Production has chosen for the 32nd Annual Conference has chosen for the 32nd Annual Conference the theme **Revitalising the Livestock Industry through Technology Transfer.** The successful animal farming is through the transfer of the discovered or improved knowledge and developed technology from the researchers including scientists and technologists to the end users that are mainly the farmers or producers.

In Malaysia, the media play an important role in disseminating news on the latest in farming technology and scientific discoveries in animal industry, but the transfer of this information is still undoubtedly slow. Extensionists and social workers need to play an active role in disseminating the technologies developed by scientists. Some government agencies do provide short courses and extension services related to animal production and farm practices. However, there are many instances that the output or value of the research has been underestimated and not receiving the right publicity as it deserves. Farmers, scientists and academics seldom meet and as such the platform of technology transfer is still limited.

The theme this year is set to look at how technology can be transferred effectively to farmers. Modern farming requires that farmers are knowledgeable and utilized efficient techniques to reduce the costs of production. It is hoped that this conference will bring government agencies, scientists and farmers together to enable exchange of knowledge and experiences.

This publication is a compilation of the scientific papers presented at the 32nd Annual Conference of the Malaysian Society of Animal Production. It is hoped that the information contained herein will contribute towards the knowledge required to implement the activities to enhance the development of the Malaysian livestock industries.

Editors

The organising committee would like to thank all participants for the Abstracts submitted. However, due to the time constraint, it was not possible for the Editors to edit all the Abstracts thoroughly. The Editors are not responsible for any errors in the published Abstracts.

REVITALISING THE LIVESTOCK INDUSTRY THROUGH TECHNOLOGY TRANSFER

A.J. Aziz, M.A. Abu Hassan and L. Mokhdzir
Department of Veterinary Services Malaysia, Putrajaya.

Current Scenario of the Livestock Industry

The livestock industry in Malaysia has grown significantly averaging 5.4% annually for the past decade. In 2010 the industry produced RM 11.26 billion of total ex-farm value of livestock products, contributed by RM 5.77 billion of poultry meat, RM 2.36 billion of eggs, RM 2.07 billion of pork and RM 1.04 billion of beef, mutton and milk. Although poultry and swine dominate the local livestock production, having achieved more than 100% self-sufficiency (SSL), the SSL's for ruminant products are below 30%. Consumption of livestock products in Malaysia has shown a steady increase with rapid growth of the economy and rising household income. Beef consumption in 2010 was 162.37 thousand metric tons, a 46.8% increase from 110.6 thousand metric tons in 2000. Poultry meat consumption was 1,013.08 and 635.21 thousand metric tons in 2010 and 2000 respectively, registering a 59.5 % increase. Egg consumption increased by 48.7% from 5,725 million eggs in 2000 to 8,525 in 2010. Milk consumption in 2010 was 1,373.17 million liter LME (liquid milk equivalent), a 30.8% increase from 1,050.6 million liter LME in 2000.

Malaysia relies heavily on the importation of live animals, beef, mutton and dairy products to meet the demand for domestic consumption and the processing industry. The value of imports of these products in 2009 was RM 6.61 billion against export value of RM 4.88 billion, registering a deficit in trade balance of RM 1.73 billion. The major import items were dairy products (65.7%), meat and meat products (31.2%) and live animals for slaughter and breeding (3.1%). In addition, Malaysia also depends on imports for animal feed ingredients, with a total value of RM 5.3 billion in 2009. Malaysia also exports animal feeds valued at RM 538.6 million in the same year.

Challenges and Opportunities

Although the livestock industry has indicated positive growth in all sub-sectors for the past decade, it has not reached its real potential in terms of productivity, value creation and competitiveness. Technical parameters such as breeding performance, growth rates, feed conversion, losses due to diseases and mortalities, has not shown much improvement especially in ruminant production. These factors contributed to low efficiency, resulting in low yield and high production cost, thus portraying unfavourable economic and non-competitive business opportunities to investors. On the other hand the poultry industry has undergone transformation successfully into an industrial, modern, precise and highly competitive integrated system enabling consumers to enjoy relatively cheap poultry products. However having achieved total self-sufficiency level (100% SSL) since 1983, the industry has not been able to move further to a higher stature towards becoming a significant player in the global market. Analysis done by Department of Veterinary Services (DVS) summarised that the industry at this stage is 'trapped' in its comfort zone and needs new impetus to grow bigger. The call and push for exports has not shown positive result mainly due to high product recovery costs contributing to higher production cost of processed products.

Significantly, the industry loses out in price competitiveness to other global players although at the farm level it is highly competitive.

Challenges and opportunities to the industry are not only about production, productivity and competitiveness occurring within its internal environment but are also influenced by its PESTEL (Political/Policy, Economy, Social, Technology, Ecology and Legal) environment both in the domestic and global scenarios. The government initiatives in transforming the Nation towards 2020 such as The New Economic Model (NEM), National Key Economic Area (NKEA) and Government Transformation Program (GTP) have direct and indirect implications to the livestock industry. The total contribution of the livestock industry estimated at RM67.6 billion to GNI (Gross National Income) in 2010 should be doubled or tripled with the right initiatives and impetus. New approaches, strategies and impetus to transform the industry along the value chain have to be redesigned not only to increase productivity and farm output but also to strengthen competitiveness while managing risk, so there is sustained growth. Contribution to GNI, employment, per capita income, inclusivity and food security should be of concern to all players and stakeholders in the industry.

The political and policy instruments indicate the direction of the nation, setting further its economic and social development. With modernization, rapid economic growth and prosperity, demand for food will increase not only in terms of quantity, but also with regards to quality, safety and halal. The changing patterns of demography, consumer behaviour and their access to information require the industry to be more responsive to social awareness, consumer needs and rights, in providing products and services. Consumers are not only concern with the food they purchase, but also to other factors implicated in the production process such as the origin, the ecological effect, the welfare of the animals and legal matters. Thus, their concerns require precise answers.

Research and Innovation

The livestock industry has evolved during the past decades through innovations in product, technology, system, method, models, process and services. Capital and knowledge farming practices and downstream industries, together with rapid adoptions and adaptation of new technologies have generate higher growth rate and raise the returns gained from productive activities along the value chain. However only the poultry has undergone changes from subsistence to modern, industrial and integrated system, enabling the production of relatively cheap and stable price of meat and eggs. Majority of ruminant livestock owners are still practicing traditional farming owning small herds with low input and minimal technology application. They generally lack or inaccessible to resources, market, knowledge and technology. Although technologies may have been developed, some major concerns of ruminant farmers in areas such as animal nutrition, breeds and breeding, disease control and husbandry still remains and often expressed by many because of its direct effect to farm profitability. The gap between traditional livestock farming and the successful modern practices are huge and need to be rectified especially through research and innovation.

Innovation has been given much emphasis by the government in all spectrums of the nation. Research being one of the means of creating innovations need to be revitalised and realigned so as to provide a great impact to the livestock industry. Research should be redirected and refocused strategically into 3 main purpose; solving immediate problems, providing effective solutions, and advancement of science for the future. This could be done through strategic alliances of all stakeholders dealing with

research and innovations such as government agencies, private institutions and professionals. Integration of various disciplines in research is also important to attain more holistic results. In the absence of critical mass and limited resources, research in precision livestock production using multi-disciplinary approach is the way forward.

Technology Transfer

It is important for the livestock industry to move into the knowledge-intensive upstream and downstream activities, such as product and services development as well as using ICT along the value chain. Livestock science, technology and farming practices have also undergone substantial changes and redefinition. Enriching all activities with knowledge also means developing new knowledge-intensive livestock industry. As livestock farms continue to evolve in size and scope, producers will acquire informational and business relationships more closely with professional service providers who are expected to understand and embody these relationships in their services. Professionals in animal health, animal production and scientists, being an integral part of livestock production need to keep pace with new developments not only on science and technology but the whole spectrum of the industry, both its internal and external environment.

The success of technology application by the livestock industry depends upon the linkages between research, extension agents (including professional service providers) and entrepreneurs. The linkages not only provide information flow to the clients but also to receive information and feedback from them. The bond between all parties in the process develops trusts and favourable working condition, hence a success in the technology transfer. Research-extension-client linkages need to be strengthened to incorporate the integration of technologies for successful adoption by the client. Further to the success of technology transfer to the client is the demonstration effect that could be emulated by other entrepreneurs. DVS has successfully developed a Veterinary Extension Services (VES) module based on integration of various disciplines and technologies. VES is a proactive approach focus towards developing potentially commercial entrepreneurs. The successful farmers and entrepreneurs become the focal points in providing the demonstration effect for others to adopt. The revised VES manual was recently launched to provide clearer representation of the research-extension-client linkages and to achieve the 2020 target of raising entrepreneurs income to RM4,000 per month.

Conclusion

The livestock industry must use resources in a sustainable and optimal ways; meet the market with innovations in products, services and delivery systems that recognize consumers' needs and rights. The industry should explore various options, innovations and experiences that have been accumulated within the country, region and other developed nations. The application of scientific knowledge, research and innovations along the value chain is vital to elevate the industry to a higher level of achievement. The livestock industry should be developing along the aspiration of achieving a high income society and developed nation by 2020.

PERSPECTIVE ON GHG CONTROL IN LIVESTOCK SECTOR FOR REVITALIZING LIVESTOCK INDUSTRY

Junichi Takahashi*
Professor, Graduate School of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido Japan 080-8555.
*E-mail: takeshige@obihiro.ac.jp

Climate change attributed to global warming induced by increase in greenhouse gases (GHG) emission influences the sustainability of livestock industry. The greenhouse gases emissions from livestock farming are methane (CH_4) and nitrous oxide (N_2O), though carbon dioxide (CO_2) contributes almost half of total greenhouse effect. Rumen CH_4 emission can account the biggest anthropogenic source. The abatement mechanism of rumen CH_4 emission may be divided to direct and indirect suppression to methanogens in the rumen. The most significant strategy to mitigate ruminal CH_4 emission indirectly is to promote alternative metabolic pathway to dispose of the reducing power, competing with methanogenesis for H_2 uptake. Prebiotics and probiotics have been innovated to mitigate rumen CH_4 emission instead of ionophores in respect to food safety. They are mostly propionate enhancers which consume metabolic hydrogen (H_2) compete with methanogens and abate rumen methanogenesis in indirect manner. Assimilate nitrate reduction in the rumen which shows strong oxidation-reduction potential is effective to mitigate rumen CH_4 emission. However, relatively higher reducing ability of nitrate reducing bacteria than nitrite reducing bacteria induces nitrite accumulation in the rumen. Successful CH_4 emission can be mitigated without toxic nitrite accumulation due to suppression of nitrate reduction when L-cysteine is simultaneously administered (1). Furthermore, *Escherichia coli* modified genetically has been developed to promote nitrite reduction abating ruminal methanogenesis (2). Recently, dissimulate reduction of nitrate with *Alcaligenes faecalis* have been attempted as another option of the safe mitigation of rumen CH_4 emission using nitrate without toxic nitrite accumulation. One of protease-resistant antimicrobial substances (PRA) has been isolated from *Lactobacillus plantarum* as a direct suppressor of rumen methanogens (3). The suppressing effect of PRA on rumen methanogenesis were examined using the *in vitro* continuous methane quantification system.

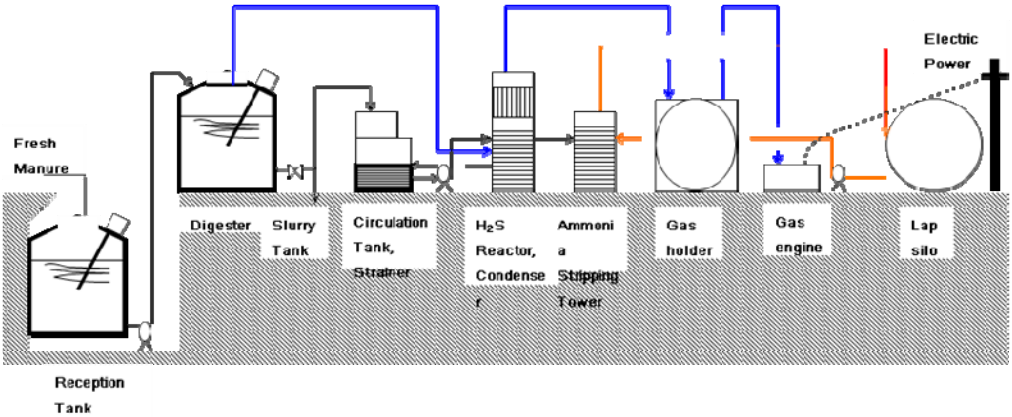


Figure 1. Plant system of ammonia stripping.

The possible use of agricultural biomass such as non-edible parts of crop plants and animal wastes was proposed as a renewable energy and nitrogen sources. The ammonia stripping from digested slurry of animal manure in biogas plant to apply three options of nitrogen recycling to mitigate N_2O emission. Figure 1 shows the plant system of ammonia stripping from digested slurry of cattle manure in biogas plant. Figure 2 shows biogas system addressed biomass to local society.

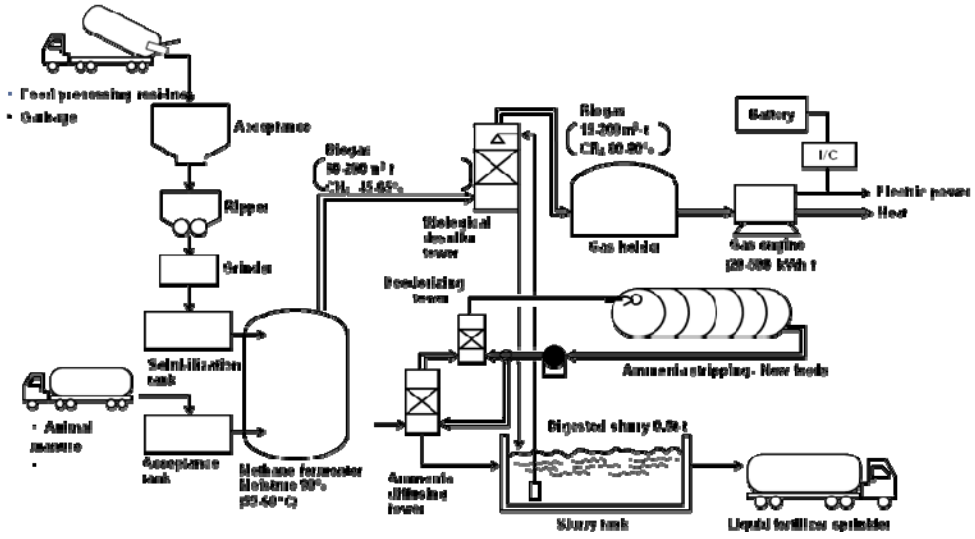


Figure 2. Biogas system addressed biomass to local society.

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THE ROLE OF DEMO FARM TO DEVELOP SMALLHOLDER DAIRY FARMING

Rochadi Tawaf[1*] and Aldi S. Surianingrat[2]
[1]Economics Laboratory, Faculty of Animal Husbandry, Padjadjaran University, Bandung, Indonesia;
[2]Sahabat Cipta Foundation, Jakarta, Indonesia.
*Email: rochadi_tawaf@unpad.ac.id

Introduction

Koperasi Peternak Sapi Bandung Utara (KPSBU) as a dairy cooperative had been established since 8th August 1971 with work areas in several districts in Northern Bandung, West Java. KPSBU has 7,000 members consisting of 5,178 active dairy farmers. The KPSBU member farms are generally small sized with 1 to 4 cows/ household. The average milk production capability is about 10-15 liters/head/day and the total milk production is about 120 tons/day. KPSBU is one of the biggest dairy cooperatives in the West Java province.

Sub district of Ciater is one of the extension working areas of KPSBU. Since 2007, dairy farming has been introduced in this area. Previously, this area was an area of tea plantation business and had a small number of dairy farmers. However, in the last five years, the area has developed with dairy cow business of various sizes due to the development impact. However, its development is still far from what was expected. The problem is mainly caused by the less intensive development of dairy farmers. Therefore, the cooperative was concerned to develop this region into one of the dairy production centres in the working areas of KPSBU so that it will achieve increased production, productivity level and income through use of innovative demo farms. This study aims to study the role of demo farm in developing smallholder dairy farmers in the Ciater area.

Method and Analysis

The study used the survey method and Focus Group Discussion. Sample selection for this study was by simple random sampling by which 61 farmers (27.11%) were selected from total population of 225 (sampling frames). The selected respondents were grouped into 2 strata as follows: Small Size (1-4 cows/household) and Big Size (more than 5 cows/household). The financial analysis was used to analyze the data collected for existing business conditions of famers in a year. Descriptive analysis was carried out to explain the resulting data

Result and Discussion

Dairy Farmers Characteristic

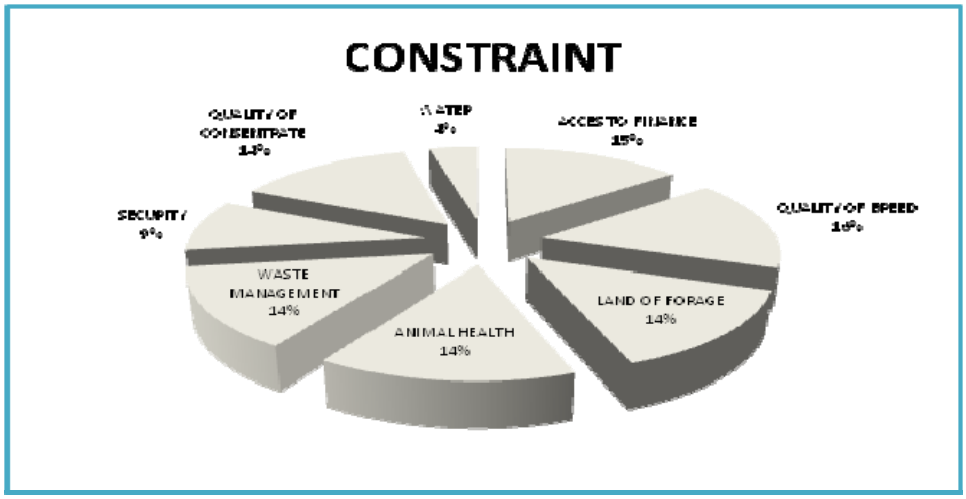
The dairy farms have developed in Ciater area in accordance with the supporting climate on high plateau. The education level of dairy farmers is mostly until elementary school (73%); the education levels of the others were junior high school (11%), senior high school (11%) and university (5%). The average milk production of the dairy farmers in Ciater is still low compared to the KPBSU dairy farmers in Lembang. In 2009, the average milk production was not optimal (6.8 liters/head/day); this can still be improved. Nevertheless, in 2010, the average milk production in this area had increased by about 35% from the previous year production (9.2 liters/head/day).

The dairy farmers can derive forage from agricultural or land waste products and rice straw from paddy field areas in North Subang area. During the rainy seasons, farmers usually collect forages from 5-10 km around the location of the stable. They get the forages for free and only pay for the transportation cost from the collection point to the stable. Meanwhile, during dry seasons the farmers will look for forages at further locations, 15-30 km, and automatically the transportation cost become more expensive. The farmers use concentrates mainly of low quality (crude protein is only about 8-9%); these are priced IDR 1,500/kg and are produced by KPSBU. During the dry season when forage is limited, farmers tend to increase the amount of concentrates given. Most of the farmers provide not only forage and concentrates, but also additional feed from the byproducts of tapioca mill industry and waste of tofu, waste of cassava, rice bran and the waste of the beer industry. Some of the raw material of the feed is imported from outside of the area regency.

The Dairy farming business in Ciater Area is efficient, because R/C>1. Nevertheless, the business with 3 heads of lactation cows in Small Size farms (R/C = 1.37) is more efficient than the Big Size farms (R/C = 1.03). This is caused by the structure of dairy population in small size business being better than the big size business.

The Constraints of Dairy Business

From the results of field study several problems faced by farmers in conducting the dairy activity were identified and may be seen in the graph below:



a. The low productivity of milk cows owned by farmers may be partially attributed to their low genetic quality (breed), 16 %. However, how to provide stock replacement of good breed and suitable to the farmers' needs is a question. This problem is quite complex because, in general, farmers do not raise calf.
b. Capital is one of the constraints in dairy farming. About 15% of the farmers needed capital in the form of credit with low interest (3-5%), like credit programs from government.
c. Dairy health management during the time is supported by KPSBU officers. Approximately 14% of Ciater farmers experienced animal health had become a constraint to their activity because of lack of knowledge in this area and unavailability of officers serving farmers 24 hours to treat their cows.

d. Low feed quality. The availability of sufficiently high quality of raw feed material that can satisfy the needs corresponding with production level.
e. Management of waste are not well organized among farmers. Many of the farmers do not have any knowledge on how to treat dairy waste.
f. Security factor is still a constraint for 9% of the farmers; this is more on the loss of their milk equipment.
g. The location Ciater, which is an area of hot water containing sulphur, is also an obstacle for 4% of the farmers. Farmers require funds for purchasing water.

Willingness of Farmers on Capacity Building

In preparing the draft of Demo Farm (DF) model and Dairy Support Centre (DSC), this study analyzed the willingness of farmers to follow the required training. The results of the analysis are as in the illustration and as follows:

a. Most of the farmers (37%) desired that the capacity building be in the management of dairy farming. The scope of this dairy farm training includes: management of breeding and reproduction, feeding, cattle raising management, housing and post harvesting.
b. Animal health (25%): including diseases control (preventive) and treating diseases (curative).
c. Waste management (23%): including composting process, vermication and its use as energy source (biogas).
d. Group management (15%): including managing group as cooperative members.

The result of these respondents' willingness hopefully can be used as material syllabus in developing Demo farm. The road map for the stakeholder dairy farm explaining the function and role of each institution involved is as follows:

1. KPSBU will guide the farmers and groups, coordinating with government and technical implementation unit (inseminator or veterinarians) in the field.
2. Group of Farmers: administrative and coordinate with KPSBU in guiding the farmers and coordinate with the government and the inseminator and veterinarians.
3. Government will guide KPSBU, farmer groups and dairy farmers as well as coordinate with inseminators and veterinarians.
4. Inseminator or veterinarian as operational technical officer of KPSBU in DF/DSC level will give services to the farmer groups and dairy farmers and coordinate with the government.

The Role of Demo Farm

As mentioned, the field study identified several constrains in the current dairy farming situation in Ciater. These constrains were identified at farm levels as well as in the business supply and value chains. At farm level farmers experienced low productivity, poor animal health condition and dairy cows of low quality breed in general. On the other hand, the study also identified constrains in the quality of dairy concentrates (of very low nutrition) and trading practices in the livestock market resulting in relatively high cow prices. In addition, in the milk supply chain, the fresh milk currently lacks a uniform reference standard in the collection and milk payment chain, which is essential to communicate, understand and improve the interest of each stakeholder.

Dairy farmers thus operate in a poor infrastructure and as a result have in general low marketing incentive and motivation to develop their farming enterprise. In

order to address this lack of motivation, it is essential that any effort to develop the dairy farming enterprises in the Ciater area does not focus only the farmers, but also addresses the constraints in the business and value chains. In this study the recommended interventions at farm level have been identified and may be dealt with demonstration, required facilities/organization and training method.

In order to reach a high demonstration impact among the dairy farming community in Ciater, it is recommended to establish several demonstration farms close to the dairy farming communities. It is proposed to establish one Demo-Farm per village or milk collection point. This will enable all farmers to participate easily and actively in the demonstration farm scheme meant for training and demonstration activities.

As shown in the field study, 95% of the farmers own less than 4 dairy cows. Consequently the demonstration and training needs to focus to this group by demonstrating a farm unit of 3 dairy cows and young stock. Demonstration units with 10 cows should be limited to a maximum of two, while there is no location identified for a larger demo-unit.

The role of establishing demo-farms to develop smallholder dairy farms were: (1) very effective way to show farmers improved farming techniques, (2) key facility for meetings and discussion points for all stakeholders, (3) ideal tool to enhance confidence and relationship among the stakeholders, and (4) seeing is believing or learning by doing.

Farm models

Three different farm models studied which varied in size to accommodate 3 up to 25 dairy cows were studied. The differences are evident mainly in the way dairy cows are kept. Tie-stall is chosen for the smallest farm type because it is easy to operate. Cows are permanently tied and do not need to be collected for milking. A small lecture area is included in this demo-unit to facilitate training activities. All demo-units include a biogas unit and a manure storage system, which are important in relation to environment management. For the farms that have to accommodate 10 up to 25 cows, a free walking stable is preferred. This design requires the farmer to collect the cows for milking, but on the other hand cows can walk freely, which has a significant advantage for the farmer in detecting cows in heat. An important aspect in the design is the accommodation for young stock. The rearing of replacement heifers should be promoted, since breeding and rearing are essential for improving the herd milk production capacity. In the different models it is assumed that the farmer keeps the same number of cow and does not increase the number of dairy cows (stable herd situation). In case the farmer wishes to increase the number of dairy cows he is obliged to increase the stable capacity.

Another significant difference is the method of milking. At the S1 type no investment is made in milking equipment, other than some basic milking utensils, because there are only a few cows. Assuming a farmer can milking a single cow in 5 minutes it will not take him longer than 25 minutes for a milking session, which is well within the recommended maximum time of 45 minutes to finish milking.

Table 1. Models of Dairy Demo Farm

Items	S1 MODEL	S2 MODEL	S3 MODEL
Dairy cows	3	10	25
Calves (0 – 3 months)	1	2	6
Young stock (4 – 6 months)	1	2	6
Young stock (7 – 12 months)	1	2	6
Heifers > 12 months	1	2	6
Stable type	Tie-stall w/ rubber mats	Free walking Raised cubicle w/ rubber mats	Free walking Raised cubicle w/ rubber mats
Milking method	Hand Milking	Mobile milking machine	Fixed vacuum line with 2 buckets
Milk cooling at farm	none	none	None
Feed gate	none	Open feed rail	Self locking feed-gate
Milk - Treatment box	none	yes	none
Manure system	Biogas	Biogas	Biogas

Conclusions

1. The demo farm was built in accordance with the desire or expectations to expand its business. Interventions at farm level are identified and deal with demonstration, required facilities/organization and training method.
2. The demo farms were built close to the dairy farming communities. It is proposed to establish one Demo-Farm per village or milk collection point. This enables all farmers to participate easily and actively in the demonstration farm scheme of training and demonstration activities
3. The role of establishing demo-farms to develop smallholder dairy farms were: (1) very effective way to show farmers improved farming techniques, (2) key facility for meetings and discussion points for all stakeholders, (3) ideal tool to enhance confidence and relationship among the stakeholders, and (4) seeing is believing! Or learning by doing.

Acknowledgement

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STRENGTHENING KNOWLEDGE-SYSTEMS LINKAGES OF VETERINARY EXTENSION SERVICES IN MALAYSIA

M.Z. Azmie, M.A. Abu Hassan, A.K. Sulaiman, L. Mokhdzir and E. Azlan
Department of Veterinary Services, Putrajaya, Malaysia
E-mail: azmie@dvs.gov.my

Traditionally, agriculture extension has always been linked between research-extension-farmers system to enhance integration of science and technology into farming systems. The Department of Veterinary Services (DVS) has introduced an innovative veterinary extension services (VES) two decades ago and recently held its 21st Veterinary Extension Annual Conference in Seremban. It was introduced in mid-eighties following the formation of a task force to accelerate the transformation and commercialization of the livestock industry through the transfer of knowledge, skills and technology.

Significant progress has since been achieved by the livestock industry from an ex-farm production value of RM 2.28 billion in 1988 to about RM 11.26 billion in 2010. The rate of technology uptake in this sector has been remarkable and may be seen in farm mechanization, such as milking machine, electric fencing, fodder chopper, feed baler and forage harvester or in farm automation, such as in water drinker, ventilation, lighting, egg collection, egg grading or in the use of information technology at the various stages of its production or in the farming system from feedlot, cattle integration, closed house or waste treatment system. Similar progress were observed in the use of new technology along the value chain from refrigeration, storage, transportation, processing and traceability as can be seen at agricultural shows such as MAHA and the biannual Livestock Asia Expo and Forum.

VES recognized the uniqueness of each partner's needs and priorities. Introduction of any changes in method, system or technology will bring with it a significant risk to them which are often ignored or underplayed by the proponent of the new technology. VES provide advice and second opinion to partners in going through the change processes. Partners will be guided through the process of trial and error in the adoption and adaptation of new method, system or technology to arrive at an optimal option for his unique operation or situation.

VES is a unique innovation developed through a combination of four disciplines after an extensive international search. VES has taken proactive veterinary services from Preventive Veterinary Medicine while recognizing the importance of farm economic through the use Gross Margin Analysis. Extension methodology is another important discipline in VES. Statistical analysis as a basis of an objective evaluation to have sound advice backed by a veterinary information systemand many modern management tools. This innovation has taken the wisdom from experiences of many countries including Germany, Netherland, United Kingdom, and New Zealand and from Malaysia particularly from Universiti Putra Malaysia. While this innovation was introduced in mid-eighties it was documented as a manual in 1993. In 1995 during the 17th MSAP Congress the initial progress of VES was reported (1).

DVS, over the last 21 years observed many achievements by our partners. The initial objectives of VES are in instilling Good Animal Husbandry Practices (GAHP), entrepreneurship and commercialization of livestock farming, which has since been

adopted by the Ministry of Agriculture through its *Agriculture, is Business* slogan. DVS involvement into the animal product processing industry through Veterinary Health Mark (VHM) accreditation has laid a foundation for a vibrant value added activities contributed toward the enlargement of ministry's function to include agro-based industry.

The positive impact of VES on the livestock industry was brought about through the intimate relationship of DVS officers with our partners. A structured interaction of scheduled and planned visit in providing the proactive veterinary services has helped to establish that relationship. Messages for change were brought to our partner individually or through the group activities such as training, visit, field day or farmers meeting. Messages for change were further reinforced through public awareness campaign. DVS acknowledged it limited resources, thus offered VES to selected partners and by leveraging on the demonstration effect to influence technology transfer to wider groups of clients.

The launching of second edition VES Manual will guide us through to year 2020 with targeted client income of RM 4000 per month. The advent of new media offer moreopportunities in engaging with our partners. While effective technology transfer will require closerlinkages between the researcher, consultant, expert, resource person, extension officer with our partners. Research and innovation will be focused more toward solving immediate problem and providing effective long term solutions. The integration of knowledge, information, technology and resources need to be adjusted to suit each partner's unique situation. Greater employment of creativity and innovations in product, technology, system, method, models, process and services through knowledge-based, capital intensive animal yet sustainable animal industry will continue to challenge us throughthe next decade.

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ISSUES AND CHALLENGES IN COMMERCIALIZING NEW LIVESTOCK TECHNOLOGIES

A.R. Azizan[1]*, M. Fiona Naqiah[2] and C.M. Nurul Akmal[2]
[1]Strategic Livestock Research Center, MARDI, GPO Box 12301, 50774 Kuala Lumpur, Malaysia;
[2]Business Development Unit, MARDI, GPO Box 12301, 50774 Kuala Lumpur, Malaysia.
*Email: achan@mardi.gov.my

Abstracts

Today agriculture industry throughout the world is rapidly transforming into a commercialized entity. This inevitable phenomenon is due to a number of factors such as the rapid growth of the world economy, market liberalization, rapid urbanization and rapid increase in demand for food. As economies grow, there is a gradual movement out of subsistence food-crop production to a more diversified market-oriented or commercialized production system. From the research institution point of view, the process of commercialization requires a paradigm shift in agricultural research priority setting and the methodology of transferring new technology to the targeted group. Currently, new technologies are transferred and commercialized through licensing, consultancy and leasing mechanisms. The most critical aspect about commercializing new technology is getting or ensuring it to produce the expected output (performance) in a business environment. In order to ensure this, all new technology must be evaluated, up-scaled, fully tested and packaged before it is offered for sale. Other issues and challenges are getting the most suitable recipient company (licensee), after-sale (post licensing) services and commercialization procedures that must adhere to governmental regulations. Currently, the Business Unit of MARDI has been able to commercialize 18 technologies which have generated income of about RM 2.7 million to MARDI in the form of licensing fee and royalty payments.

Introduction

Agriculture today is not only about farming- it's a business. Agriculture industry today is rapidly transforming into a commercialized entity. This inevitable phenomenon is due to a number of factors such as the rapid growth of the world economy, market liberalization, rapid urbanization and rapid increase in demand for food. As economies grow through liberalization of domestic markets, there is a gradual movement out of subsistence food-crop production to a more diversified market-oriented or commercialized production system. Today's global economy, business competitiveness is not only dependent on production efficiency but also in terms of product quality, safety, and pricing and also biodiversity preservation. It is well acknowledged that to achieve these factors it require the uptake of modern technologies. It is well established that research and technology development have been the foundation of impressive productivity gains in the agricultural sector. Thus the challenge ahead for a public research institution like MARDI is to continue producing and transferring technologies that are required by the agriculture sector so as to be able to further improve the competitiveness of the agriculture business in Malaysia. The major transformation for MARDI to undergo in order to fulfill this mission is to emphasize more on the generation of "demand-pull" technology and transferring of technology through marketing and sales.

Technology Generation

MARDI's technology generation process is closely modeled on the Stage-Gate model developed by Robert G. Cooper in 1986. The Stage-Gate model sees technology innovation as a clearly define process. According to Cooper (1986), the model's objective is to increase the quality of product/technology innovation by focusing on the process rather than the product. By dividing the technology generation process into sequential phases, overview can be maintained and when necessary corrected. The result is that inventions get as fast as possible to market by eliminating unnecessary activities. Managing issues at an early stage maximize the chances that an invention becomes a commercial success.

MARDI's technology generation model in the broad sense involved two (2) distinct organizational entities namely the research and technology transfer wings. The research wing or centers carry out the task of generating new technologies or inventions where as the technology transfer wing mainly deals with technology transfer activities which includes sales and services of packaged technology. In MARDI's technology generation model the innovation process is sub-divided into six (6) stages where work is performed by various multidisciplinary teams (Table 1). The gates in this case are actually the evaluation and monitoring exercises within the stages to evaluate and monitor the progress of the inventions/innovation process.

Table 1. MARDI's sequential model for technology generation and commercialization

Stage	Activity	Gate	Outcome
Idea Creation	Researchable ideas of value	Head of Research Program at Research Centers	Research Project Title
Project Proposal	Literature review and complete research project proposal write-up	Technical Expert Committee at Research Centers	Project Proposal approval for funding
Basic Research	Research Activities	Science-Fund Technical Evaluation Committee	Proof of concept (POC)/Invention
Technology Disclosure	Research findings presentation	MARDI's Technology Management Committee	Endorsement for IP registration & commercialization evaluation
Technology Development	Technology screening and evaluation	BU's Technology Evaluation Committee	Pre-commercialization or technology packaging
Technology Packaging and Marketing	Business model and plan development. Business matching and negotiation	Targeted cliental such as GLC, PLC, SMEs', limited and private limited companies.	Licensing, J/V or spin-offs companies

Technology Marketing and Sales

The most critical aspect about marketing and selling of new technology is getting or ensuring it to produce the expected output (performance) in a business environment. This task has been empowered to the Business Development Unit of MARDI. Our main functions are to develop MARDI's research findings into value propositions and ultimately to market or commercialize these findings. It is important to note that R&D findings will remain intangible as long as they are not commercialized. Therefore, our efforts are mainly directed towards commercialization of MARDI's research findings. In order to carry out our task effectively, we categorized our Business Development Unit activities into 3 main thrust areas namely; i) Screening, evaluation and packaging of technology ii) Pre-commercialization of technology and ii) Marketing and sale of packaged technology

Basically, the first thrust area allows the identification of proven technologies either for pre-commercialization or commercialization purposes in the case for in-complete and complete technology, respectively. New technology declared by the research centers are evaluated for completeness through the plan innovation model (PIM) matrix. In aspect we look at the technical production or process method, physical and non-physical needs, emotive and economic values, competitive advantage and marketing strategies. When all these conditions are met then only the financial evaluation will be carried out. The Projected cash flow analysis, partial and full budgeting techniques are some of the methodologies that we used to evaluate the financial benefits of the new technology. Complete technologies with good economic and financial potentials are then packaged up for sale to interested parties.

The second thrust area concentrate on the task of technology up-scaling, technology incubator system and franchise development. Incomplete technology that needs to be up-scaled are either processed for pre-commercialization funding or further tested in MARDI's technology incubator system. Technologies that successfully "graduated" from this program are then packaged up for sale. The third thrust area actually deals with marketing and sale of packaged technologies through consultancy, leasing and licensing.

As to date MARDI has given out 18 licenses to various companies for 18 technologies encompassing livestock and feed production, agricultural machineries and equipments, plant varieties, food processing and fertilizer production. Out of the 18 technologies, 10 are still active and contributing royalty payments. MARDI's has collected about RM 1,925,000 (Ringgit Malaysia one million nine hundred and twenty five thousands) from technology disclosure fees (TDF), technology licensing fees (TLF) and management fees (MF). MARDI has received about RM 775,003 (Ringgit Malaysia seven hundred and seventy five thousand and three) in royalty payments from these technology licensing.

Issues and Challenges in the Commercialization of New Livestock Technology

It has been acknowledge that the future challenges in the commercialization of agriculture or livestock technology lies in the business aspect and the best practices (methods) in transferring the new invention into the nation's economy. The business challenges of pushing new technology to market in the agriculture or livestock industry are changing – it is no longer adequate to produce new inventions and hope to sell it through aggressive marketing campaign. Some of the identified issues and challenges

in commercialization of new agriculture or livestock technologies are value creation (Boehlje, 2004), suitable recipient company (licensee), after-sale (post licensing) services and commercialization procedures that must adhere to governmental regulations.

According to Boehlje (2004), the most basic business challenge in introducing any new technology is that of creating value for the customer. In other words can the customer make profits by adopting this new technology? We must make sure that the new technology must produce the expected output (performance) in a business environment. In the case of our oil palm frond (OPF) technology, the appreciation of our currency and logistical issues of collecting OPF from the field has resulted in creating less value to our licensee company. A decade ago the ex-factory selling price of OPF was RM 350 per metric ton whereas now the price has gone down to RM 290 per metric ton because of our currency has appreciated greatly against the US dollar. The 17% dropped in selling price of OPF feed makes the project less feasible. In addition the difficulty in collecting and transporting the OPF from the fields to the factory has rendered the project less feasible due to an unanticipated rise in delivery cost of the raw materials.

The other major challenges in commercializing new livestock technology is getting the most suitable licensee company. The recipient company must have adequate funding, technical experience and total commitment to carry out the business. Newly formed company usually failed to execute the licensing agreement. A good example is a newly formed company that failed to produce our mineral lick block after this technology was licensed to them. The newly formed company had no prior experiences in producing mineral lick block and also the company was short of fund to automate production. Even though the company took a RM 2.5 million bank loan yet the project failed to sustain because of *business negligent*. The *Otosil* technology on the other hand is a successful venture where the recipient company (Choon Heng Engineering Company) is a machine fabricator company. Choon Heng Engineering Company was able to tap into their vast experience in machine fabricator work and thus was able to translate the blue-print design into workable silage machines.

Post-licensing services is another major challenge in the commercialization of new livestock technology. A good example will be the *Brakmas* Cattle technology that was licensed out to FELDA in Chuping, Perlis. The license to produce and sell *Brakmas* cattle was given out to FELDA in 2005. Until today, the project has yet to give out royalty payment to MARDI. During this period MARDI was involved in the development of the farm's infrastructures, selection and purchasing of animals and the training of farm workers. On top of that MARDI officers had to visit the farm regularly to conduct pregnancy diagnosis and to assist in the general management of the farm. All these post-licensing services were not penned in the agreement much earlier but nevertheless, these services were critically needed for the success of the project.

Commercialization of new livestock technology also faced challenges in the form of governmental procedures that need to be followed. Even though MARDI is classified as semi-government body but its entire budget comes from the government. Thus, MARDI is obligated to follow strictly to the government regulations when dealing with administrative matters including commercialization of technologies. It is rather difficult when you are actually doing business dealings with private companies but subjected to the bureaucratic procedures set by the government.

The future challenges in the commercialization of new livestock technology is to execute the best method of effectively transferring (commercializing) the new invention to the targeted group. Currently MARDI executes consultancy and licensing approaches as a vehicle for transferring new technology to its customer. Both this methods have been proven to be very successful and further more quite easy to implement and manage them. From a financial viewpoint, both consultancy and licensing only give rather limited financial gain in the short term period. It has been acknowledged that quality of the invention, market value, and appropriability mechanisms are in these approaches considered as important factors shaping the commercialization choice (Dechenaux et al, 2008; Lowe and Ziedonis, 2006, Shane 2000). New methods include creating profit sharing agreements, joint-ventures and spin-offs companies are considered to be of better choices today because they give more returns financially. According to Bray and Lee (2000), returns from spin-offs companies are 10 times more than the average annual income from traditional license, and is significantly higher than the amount usually received as a technology licensing fee.

Conclusions

The rapid growth of the world economy through market liberalization has slowly transformed the livestock sector into a more commercialized entity. Commercialization of livestock industry has been undoubtedly being supported strongly by the advent of new technological discoveries. The ways these new technologies are transferred to the customer are also changing in tandem with the commercialization of the livestock industry - from simple “give-away” to value trading. MARDI is slowly getting into the process of commercializing its technological findings through consultancy and licensing efforts. These commercialization efforts brought about many issues and challenges such as value creation, suitability of the licensee company, post-licensing services and bureaucratic governmental procedures. This issues and challenges has to be overcome and so far MARDI has been able to commercialized five (5) of is new livestock technologies. In order to remain competitive and relevant in the near future MARDI has to embark into creating more value proposition for its new technological findings and generating more revenues by creating spin-offs and joint-venture companies through these new technological findings.

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FERMENTATION CHARACTERISTICS OF SOME MALAYSIAN OIL PALM BY-PRODUCTS

M.A. Rajion*, S.S. Wong, Y.M. Goh and M. Ebrahimi
Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
*Email: mohdali@vet.upm.edu.my

The extensive oil palm industry provides an abundant supply of oil palm by-products such as oil palm fronds (OPF), trunks, empty fruit bunches, palm oil mill effluent, palm kernel cake (PKC), decanter cake (DC), and palm press fibre (PPF). These by-products have the potential to be integrated into feed ingredients of ruminants. The aim of this study was to investigate and compare the suitability of OPF, PKC, and DC as ruminant feed by measuring the *in vitro* fermentation characteristics and their effects on rumen parameters.

The *in vitro* fermentation characteristics of OPF, PKC and DC were evaluated using the *in vitro* gas production technique (1). The OPF and PKC were purchased from the Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. Evaluation of the fermentation characteristics was based on the parameters, namely total gas production, rumen pH, volatile fatty acid (VFA) production and the protozoal population. The by-products were assessed at inclusion levels of 0% (control, 100% concentrate), 15% (15% by-product + 85% concentrate) and 30% (30% by-product + 70% concentrate). The *in vitro* fermentations were performed simultaneously in triplicates using a single source of rumen fluid inoculum. Rumen fluids were collected from four rumen-fistulated Kacang crossbred goats under the same feeding regime (fed twice daily at 09:00 and 17:00 h. with 70 % commercial goat concentrate and 30 % oil palm frond) and pooled. Twenty-five ml of rumen fluid and bicarbonate-phosphate buffer mixture (1:4 v/v) were placed with 0.25g of substrate into a 100 ml gas-tight plastic syringe and incubated at 39˚C for 48 hr under anaerobic conditions. The rumen liquor pH was measured immediately after the incubation period with a Mettler-Toledo pH meter (Mettler-Toledo Ltd., England). The volatile fatty acid profile of the rumen liquor was determined using a 5890 Hewlett-Packard Gas-Liquid Chromatography (Hewlett-Packard, Avondale, PA). The rumen liquor protozoal counts were carried out using a haemocytometer, following the improved Neubauer ruling. Data were analyzed by the PROC GLM of SAS (SAS Inst. Inc., Cary, NC) with class variables including different inclusion levels of OPF, PKC and DC. Significance between individual means was identified using the Tukey's multiply range test.

Total gas productions of the 15% OPF, 15% PKC, 15% DC, and 30% DC were similar to that of the concentrate ($P>0.05$). The rumen pH of all the treatments ranged from 7.01 to 7.20. There was no significant difference in pH between the by-products and the concentrate (7.09). The high fibre fraction of the oil palm by-products probably aided in buffering, thus delaying any decrease in pH (2).

The oil palm by-products did not influence the proportions of propionate and acetate. The DC increased the production of butyric acid, while OPF and PKC inclusion at different levels did not change the butyric acid production. These results agreed with (3) who fed different inclusion levels of OPF to sheep.

The two major rumen protozoa identified were the entodinium and holotrich species. The *in vitro* fermentation of all the by-products produced a significantly higher ($P<0.05$) entodinium population than the concentrate group. The holotrich populations for the 30% OPF, 30% PKC, and 15% DC were similar. The 15% OPF (0.40×10^6/ml), 15% PKC (0.37×10^6/ml), and 30% DC (0.41×10^6/ml) produced significantly higher ($P<0.05$) holotrich populations than that of the concentrate (0.16×10^6/ml). The results demonstrated that the inclusion of the by-products in the concentrate feed significantly ($P<0.05$) increased protozoal population in the rumen environment. This suggested that the concentrate feed may have a defaunation effect against the rumen protozoa which agreed with the findings of (4) that fed goats with different inclusion levels of OPF and found that increasing the OPF inclusion level in the diet increased rumen protozoal population significantly.

The decanter cake (DC) at inclusion levels of 15% and 30% yielded similar gas production, rumen pH and VFA profiles compared to the concentrate. It is concluded that the decanter cake showed the greatest potential to be integrated into concentrate feeds.

The overall results showed that the inclusion of cheaper oil palm by-products to substitute for the expensive concentrate is highly possible and applicable, without affecting the rumen fermentation parameters. The inclusion of PKC and OPF could be an alternative to improve ruminal fluid fermentation end products in terms of protozoal population and modification of rumen pH. The present results suggest that the oil palm by-products evaluated in the study have the potential to be used as feed ingredients.

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A COMPARATIVE STUDY OF THE TENDERNESS OF LAMB LONGISSIMUS DORSI MUSCLE AT DIFFERENT RIGOR STATE

S. Sadi[1], G. Geert[2], M. Koohmaraie[3], S. Shanmugavelu[4] and A. Dzulfazly[5]

[1,4,5]Strategic Livestock Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), G.P.O.Box, 12301, 50774 Kuala Lumpur, Malaysia, [2]University of New England, Armidale 2350, New South Wales, Australia; [3]King Saud University, College of Food and Animal Production, P.O. Box 2460 Riyahd 11451, Saudi Arabia/IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, 98155, WA, USA.

*Email: sadi@mardi.gov.my

In Malaysia, meat is commonly cooked within several hours of slaughter. The obvious reason for this way of meat preparation is the association with freshness and quality. Cooking meat within several hours of slaughter means that the meat is cooked in the pre-rigor state. Meat cooked after chilled storage is in the post rigor state, and has aged to a certain extent. Generally, ageing has a large impact on tenderness of cooked meat, due to the proteolytic activity of the calpain system (1). Cooking of meat in the pre-rigor state causes strong muscle contraction. Depending on the level of contraction, meat can be relatively tender or very tough (2).

The pre-, at- and post-rigor muscles were prepared in a non-randomized blocking procedure arrangement based on position within muscle. All muscle samples were heated based on a standard cooking method for meat research purposes. Muscle samples were analyzed for cooking loss, pH, Warner-Bratzler Shear Force (WBSF) and sarcomere length. The data were analyzed using Minitab 15 and presented in Table 1.

Table 1: Means and SEM for pH, cooking loss, sarcomere length and shear force value after different cooking method of pre-, at- and post-rigor lamb *longissimus dorsi* muscle.

Independent variable	N	Cooking method and rigor state								SEM
		Pre rigor				Post rigor				
		Pre rigor		At rigor		Day 1		Day 7		
		70°C	100°C	70°C	100°C	70°C	100°C	70°C	100°C	
pH	6	6.66[a]	6.76[b]	6.24[c]	6.37[d]	-	-	-	-	±0.01
Cooking loss (%)	6	16.4[a]	34.7[b]	19.4[c]	35.2[b]	23.5[d]	40.0[e]	23.0[d]	40.0[e]	±0.37
Sarcomere length (μm)	6	1.07[a]	1.05[a]	1.20[b]	1.16[b]	1.44[c]	1.44[c]	-	-	±0.01
WBSF value (kg)	6	9.4[a]	5.4[b]	9.6[a]	7.0[c]	7.2[c]	4.9[d]	3.7[e]	3.3[f]	±0.27

[a, b, c, d, e, f] Means in the same row bearing different superscripts are significantly different (P <0.01); 70°C= Cooking to a core temperature of 70°C; 100°C = Cooking at 100°C for 3 hours; – not determined. SEM=Standard Errors of the mean.

The mean pH of the raw pre-rigor and at-rigor in this experiment is within the range of values reported by Koohmaraie et al (3), and both are increased after cooking (100°C). This result is in agreement with Shin et al. (4). The increase in pH after heating could be due to the loss of acidic groups in the meat (5). The mean cooking loss of the pre and post-rigor muscle cooked at 100°C are consistent with the findings of Abugroun et al. (6). Muscle pH also affects cooking loss (7). In this experiment the effect of pH was the dominant factor affecting cooking loss. Furthermore, the mean value of the sarcomere length in the present study is within the range of those reported by Abugroun et al. (6). It is well known that sarcomere length decreases during cooking due to protein coagulation (8) and shortening (1). In this study, pre-rigor heating resulted in a sarcomere length corresponding to 1.3 μm for raw muscle, i.e. 35%

shrinkage. Obviously, this amount of shortening is expected to cause a toughening effect.

A strong correlation (R^2 = 0.76) was observed between WBSF value of pre- and post-rigor cooked at 70 and 100°C respectively. This result indicates that the toughness level of the meat cooked to 70°C cannot be completely resolved by cooking for an extended period. Both pre- and post-rigor meat cooked at 100°C gave lower WBSF values (P<0.01) than 70°C. The most likely explanation for the tenderizing effect of long term cooking is solubalization of connective tissue (9). Furthermore, it was observed that the WBSF of pre-rigor samples cooked to 70°C was similar to that of at-rigor samples with values of 9.4 and 9.6 kg respectively. As discussed above, the level of contraction at both sampling times were such that a similar level of toughness could be expected. However, surprisingly, cooking at 100°C resulted in more tender meat when the meat was cooked in the pre-rigor state. The reason for this is unclear. We can only speculate that the pH of the cooked meat has an effect, although this effect is not obvious through, for instance the effect on cooking loss.

The well-documented effect of ageing on tenderness was evident in the present study, and this effect could not be achieved by either cooking the meat early post mortem or long term cooking. Thus, it can be concluded that preparation of pre- and at-rigor meat produces very tough meat, unless the meat is prepared almost immediately after slaughter. The explanation for the toughness of early postmortem meat is the muscle shortening that occurs during the heating process. Traditional long term cooking does improve the tenderness of early postmortem meat, however not to the extent of the impact of ageing of meat.

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FORAGING ACTIVITY OF *AERODRAMUS FUCIPHAGUS* SWIFTLET OVER OIL PALM CANOPY AND ITS EFFECT ON *ELAEIDOBIUS* SPP POLLINATOR POPULATION

M. I. Kamarudin[1*] and M. Anum[2]

[1]Livestock Technology Resources Development Division, Department of Veterinary Services Malaysia, Ministry of Agriculture and Agro based Industries, Wisma Tani, Blok Podium, Lot 4G1, Precint 4, 62630 Putrajaya;[2]School of Biological Sciences, Universiti Sains Malaysia, Penang.
*E-mail: kamar@dvs.gov.my; kamar57@yahoo.com

Elaeidobius spp weevils are important insects for the pollination of oil palm (1). The insects were introduced in Malaysia in 1981(2) and increased palm oil yield by 20%, making sustainability of the weevil population important for palm oil production. Currently, the government is actively promoting swiftlet farming for the high price of edible bird's nest (EBN). The swiftlet industry is expected to contribute substantially to the Government National Income under the Economic Transformation Program. As the swiftlet, *Aerodramus fuciphagus* that produces white EBN is an insectivore and forages over oil palm canopy, there is a concern that the bird may affect the weevil population and subsequently reduces palm oil fruits yield. A study on swiftlet diet (3) has shown that *Coleoptera* is one of the insect orders found in their digesta. Other insects are *Hymenoptera, Diptera, Homoptera, Arachnida* and several other orders. This study was conducted to ascertain order of insect's aerial presence over canopy of oil palm tree and deduce the effect of swiftlet foraging activity on the weevils population.

An oil palm plantation in Kluang, Johore was selected as the site of study based on these criteria: cooperation of the plantation owner, oil palm tree were bearing fruits and aged between 5 to 7 years, weevils were observed in bunch of male flowers, and the presence of swiftlet colony foraging over oil palm canopy. A 2-meter radius balloon was filled with helium gas and was raised over 60 meters above the ground. Five insect traps were tied at the balloon rope at height of 10 to 50 meter above the canopy (Figure 1). Insects were counted and identified by removing the traps in the morning (10.00 am) and evening (7.00 pm). The balloon was re-raised after each observation and the fresh traps were replaced. The observation was made six times in three days. The trapped insects were identified up to order level. For order of Coleoptera, the species were identified and ascertained whether the insect were *Elaeidobius* spp.



Figure 1: Setting up insect trap and the balloon with insect traps raised over oil palm canopy.

A total of eight insect orders were identified over the oil palm canopy (Table 1). The main insects were *Diptera* and *Hymenoptera*. Only few *Coleoptera* insects (5.6%) were recorded and none of these were of *Elaeidobius* spp.

Table 1: The insects identified in the evening (7.00 pm) and morning (10.00 am) over the oil palm canopy of the oil palm plantation.

Order of insects	Day 1		Day 2		Day 3		Total
	pm	am	pm	am	pm	am	
Diptera	12	13	40	15	12	6	98
Hymenoptera	1	7	15	8	4	-	35
Coleoptera*	-	-	5	3	2	-	10
Hemiptera	1	1	5	-	-	2	9
Plecoptera	1	-	1	1	-	1	4
Lepidoptera	3	1	6	-	1	1	12
Arachnida	-	1	2	1	1	2	7
Orthoptera	-	-	-	-	-	1	1
Total	18	23	74	28	20	13	176

* None of Coleoptera insects were identified as *Elaeidobius* spp.

The results of the study showed Dipteral insects were the main insects present over oil palm canopy. This may suggest that insect genus Diptera was the main feed available for the swiftlet over oil palm canopy. Although insects of the order Coleoptera were also present in a small percentage over the canopy, none were weevils *Elaeidobius* spp. Based on this finding, we concluded that the weevils were not the source of swiftlet diet and their population is less likely to be effected by swiftlet foraging in the area. Furthermore, weevils can be considered hard insect which is not preferred by the swiftlet. This might be true as swiflet has been reported to be selective in their insect's choice (3). Repetition of the same study in other sites could reaffirm these preliminary findings.

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RISK OF MYCOTOXINS (AFLATOXIN) IN FEED: REVIEW ON DISEASE APPROACH IN SHEEP

S. Gunalan*, A. Shah and M. Hisham Fariz
[1] Regional Veterinary Diagnostic Laboratory Kuantan, Jalan Sri Kemunting 2, Kuantan, Pahang
*Email: gunalan@dvs.gov.my

Sheep rearing in Malaysia has become important livestock production sector and the impact in feed contaminate with Mycotoxins impaired the sector as well as the national food production. Mycotoxins are toxic secondary metabolites produced by fungi (mainly *Aspergillus*, *Penicillium* and *Fusarium* genera) and contaminate various agricultural commodities either before or under post-harvest (1) especially at high humidity and temperatures conditions. Exposure to mycotoxins by ingestion, inhalation or absorption (through the skin) it will cause reduced animal production significantly, either due to sickness or death on human and animals. Generally, diagnosis of the mycotoxins is depending on the feed samples submitted prior to feeding and postmortem result (2).

Aflatoxin is one of the most potential carcinogenic substances (hepatocarcinogen) rated as Class 1 human carcinogen by IARC (International Agency for Research on Cancer) and also closely related to mycotoxins that occurs naturally in animal feeds and it produced by *Aspergillus flavus* and *Aspergillus parasiticus* (3). Both acute and chronic aflatoxicosis will either reduce growth rate losses to the industry. The exact mechanism by which aflatoxins reduce growth rate is not known, but the animal will shows the signs of poisoning include loss of appetite, decreased feed efficiency and immunosuppression. The aflatoxin also can decrease the availability or quantity of bile salts in the gastrointestinal tract, resulting in decreased absorption of fat-soluble vitamins (3).

The severe toxic effect of aflatoxin may include severe hepatotoxicosis, immunosuppression, reduced performances, oncogenesis, or death (4). Lamb is sensitive to aflatoxin and cause decreased performance, serum biochemical alteration, pathologic hepatic lesion, decreased immune function, and death which can be economic importance. Aflatoxins (Type B1, B2, G1, G2) produced by mycotoxins producing fungi may cause liver disease (hepatotoxic, hepatacarcinogen), carcinogenic and teratogenic effects.

Mycotoxins may also exert adverse effects on ruminal bacteria by decreasing ruminal digestion of dry matter, acid detergent fibre and starch, inhibiting the growth of rumen organisms by exerting antimicrobial, antiprotozoal and antifungal activity.

In serum, the bacteriostatic activity was lower in the lambs exposed with aflatoxin without affecting serum opsonic activity. The levels of IgG in lambs are still higher; eventhogh the phagocytosis by the neutrophils is high, and usually i*n vivo* cellular immunity was lower during aflatoxin exposure period. Aflatoxin B1 is a secondary metabolite of *Aspergillus flavus* in feedstuffs, which induce hepatic tumour and other cancer (5). When immune cells exposed to aflatoxin, the cell proliferation and antibody formation were decreased (6). In addition, aflatoxin might reduce the function of macrophages (7) that function to release various active products for the nonspecific primary defense against foreign infection agents (8). The decreased anti tumour activities by aflatoxin were mediated by decreased production TNF-α, NO, H_2O_2 and O^-_2 as the secretory mediators play an important role for antitumor effect (9).

Alteration in blood biochemical value in animal fed aflatoxin is induction of disturbance in metabolism. Plasma protein may markedly lower followed severe liver damage or prolonged dietary protein deficiency (10). And, the plasma total protein, cholesterol and urea contents were lowered in lambs fed 2.5ppm of aflatoxin for 35 days. The serum albumin and globulin levels were lowered in sheep fed 2 ppm of aflatoxin for 37 days (11) that may influence growth rate. For calcium and phosphorus also lowered in sheep fed aflatoxin as liver and kidney disorders due to disturbance in Vitamin D metabolism. Lambs fed 2.5 ppm of aflatoxin for 21 days had lower plasma calcium, phosphorus, magnesium, potassium. As the consequence of intoxication, the plasma urea and gamma glutamyl transferase activity were also reduced (4).

Lowering in the average daily gain was the most sensitive indicator of aflatoxicosis in sheep, and that the immune response which was altered could render the sheep more susceptible to infectious diseases (12). Thus, mycotoxins do play an important role in production of livestock, and prompt diagnosis must be made to identify the presence of these mycotoxins (aflatoxin) in the animal feed. This review paper mainly focuses in the adverse effect mechanism that may occur in sheep. More research needs to do in this field to determine the problem in sheep due to aflatoxin (mycotoxins).

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MASS REARING OF *MEGASELIA SCALARIS* AS SWIFTLET'S FEED

C. K. Khoo[1], E. H. Tan[2], B. H. Teo[2], A. Hamdan[2] and M. I. Kamarudin[1]
[1]Livestock Technology Resources Development Division, Department of Veterinary Services Malaysia, Ministry of Agriculture and Agro based Industries, Wisma Tani, Blok Podium, Lot 4G1, Precint 4, 62630 Putrajaya; [2]School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang.
*Email: ckkhoo@dvs.gov.my

Being an aerial insectivore, edible bird's nest swiftlet feed on a wide range of insects. Their preys include hymenoptera, diptera, coleoptera homoptera, arachnida and several other orders (1). Edible bird's nest swiftlet is originally resided in caves and preferred foraging through forest canopy (2). However, natural cave populations of swiftlet have been reported to be declining in contrast to dramatic increased colonization of many artificial new man-made houses (3). Thus, mass rearing of insects is essential as an attempt to establish and maintaining artificial swiftlet colonies for the purpose of edible-nest farming. *Megaselia scalaris*, a small yellowish-brown cosmopolitan distribution phorid was chosen in this study. These flies were routinely culture in laboratory in small scale (4). In this work, we report the establishment process of mass rearing the scuttle fly, *M. scalaris* to be served as swiftlets feed.

The mass rearing of the *M. scalaris* was performed at the insect rearing facility in Institut Veterinar Malaysia (5). The adult flies were allowed to oviposit in an enclosed muslin cloth netting chamber and were supplied with commercial diet. Oviposition were conducted for 12 hours at 26-28^{o}C. Upon completion of oviposition, all the adult flies were harvested and kept at -20^{o}C for use as swiftlet feed. The whitish rice grain shape fly eggs were found scattered all over the diet (Figure 1a). Eggs laid on the diet were subsequently transferred to the rearing tray which contained commercial diet. Rearing trays (30 trays per chamber) were then incubated within environmental chambers at 26-28^{o}C. Larvae were actively fed on the diet as early as on the first day post-transfer (Figure 1b). Pupating was recorded within 3-5 days post emergence of larvae (Figure 1c). The adult flies were recorded emerging from pupae within 7-9 days post pupating.

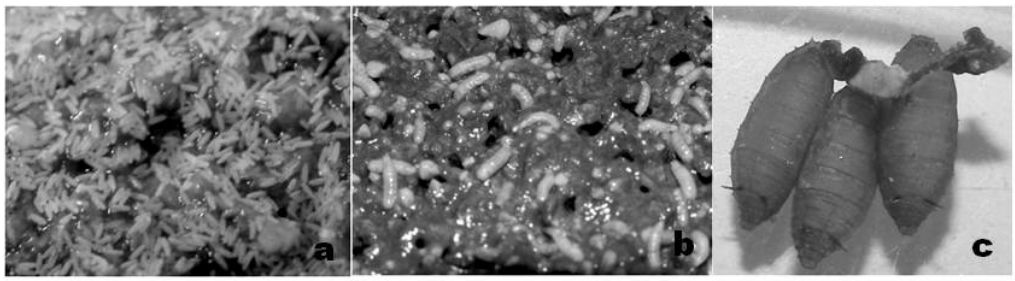


Figure 1: a) Eggs, b) Larvae, c) Puparіums

In summary, mass rearing of *M. scalaris* were successfully conducted. The development cycles of the observed flies were in accordance with the other reported studies for laboratory scale (4). Hence, potential of mass rearing *M. scalaris* as feed source for swiftlet could be exploited.

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GENETIC IMPROVEMENT IN BRAHMAN CATTLE USING ESTIMATED BREEDING VALUE (EBV)

A. Punimin*
Department of Veterinary Services and Animal Industry, Kota Kinabalu, Sabah
*Email: Punimin.Abdullah@sabah.gov.my

The purpose of animal breeding is not to genetically improve individual animals, but to improve animal populations, to improve future generations of animals (1). The most rapid progress in genetic improvement is achieved through accurate and effective selection. Genetic gains are permanent and cumulative, which means that genetic gains made within the herd will remain unless inferior bulls or cows are introduced from the outside the herd. In Malaysia, selection of animals for breeding purposes is based more on visual and physical characteristics rather than on genetic merits. This may hamper the growth of the livestock industry, as unknown genetic merits animals are being use for breeding. Animal with high genetic merit will channel the favourable genes for the desired traits and increasing their frequencies in the subsequent generations.

Sabah government through the Department of Veterinary Services and Animal Industry had initiated the genetic improvement program in Brahman cattle through the collaboration with Australian Brahman Breeders' Association (ABBA) and BreedPlan in 2008. The objective of the program, beside improvement in genotype, was to produce animals with EBV and its genotype was recognized internationally through ABBA's certification. Under this program, 120 heads of commercial Brahman cows at the Tawau Livestock Breeding Station were selected and mated with pedigree Brahman bulls with known Estimated Breeding Values (EBV) (Table 1). The cows were divided into five herds and reared solely on fertilized pasture. Two seasonally mating season of 75 days per season were used at every 6 months interval. The bull:cow ratio used was 1:25. The animals were weighed every three months. The calves were weaned when it reached the body weight of more than 150 kg at weighing program. All weight data were submitted to BreedPlan in Australia for EBV analysis. BreedPlan was an evaluation system to estimates the genetic, or breeding value of an animal using a number of measurements made at various stages of the animal's life and the performance of its relative (2).

Table 1: The EBV of the Pedigree Brahman Bulls used in the breeding program

Bull No	Estimated Breeding Value		
	200 Day Wt. (kg)	400 Day Wt. (kg)	600 day Wt. (kg)
5179	+20	+31	+38
5181	+25	+39	+45
5564	+32	+47	+54
5663	+26	+29	+47
5672	+17	+31	+25

The first batch of calves was born in 2009. There were 71 calves (28 male and 43 female), which were survived and measured for their EBV. Table 2 shows the mean EBV for the male and female calves according to their sire. The mean EBV for the male calves at 200 day, 400 day and 600 day was +16, +23 and + 31 kg respectively. While, the mean EBV for the female calves at 200 day, 400 day and 600 day was +17, +24 and +32 kg respectively. The mean EBV for calves sired by bull 5564 was the

better performers among calves from the five sires used. This was expected as bull 5564 had the better EBV compared to the other bulls.

Table 2: The EBV for calves born in 2009 according to their sire

Sire No.	n	Male			n	Female		
		200 Day	400 Day	600 Day		200 Day	400 Day	600 Day
5179	5	+13	+17	+25	8	+13	+18	+26
5181	10	+14	+21	+28	9	+14	+21	+28
5564	4	+24	+36	+42	6	+23	+34	+42
5663	5	+19	+23	+35	14	+18	+23	+35
5672	4	+16	+24	+26	6	+19	+29	+34
Total	28	+16	+23	+31	43	+17	+24	+32

Present-day progress in livestock performance is largely driven by genetics (3). The current genetic improvement program, which solely based on 200 day, 400 day and 600 day traits had shown genetic improvement in the Brahman cattle in Sabah, and potential for further development with the inclusive of other traits which economically important to the local beef industry. Economic orientation of genetic improvement with consensus by many industry participants as to its validity is an alternative to ownership for a progressive breeding industry (4). Identification of the additive genetic merit of livestock is required to make effective decisions about replacing breeding animals and, therefore, improving subsequent generations. The traits of paramount interest were usually associated with a direct or anticipated future impact on revenue or cost of production. The production of cattle with EBV from the Brahman's genetic improvement program, hopefully, will accelerate further the beef industry in Sabah. Cattle production will reach market weight much earlier and farmer's benefits from shorter rearing period and cost of production could be reduced.

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INFLUENCE OF DIETARY MOLYBDENUM, SULFUR AND ZINC ON COPPER METABOLISM IN SHEEP FED PALM KERNEL CAKE BASED DIETS

M. Ivan* and A.R. Alimon
Institute of Tropical Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor
*Email: mivan@putra.upm.edu.my

Palm kernel cake (PKC) is a by-product of the palm oil industry; approximately 3 million tons is produced in Malaysia annually and exported mostly to Europe for use in diets of cattle. It is a good feed for ruminants, but because of its high content of Cu (11 to 55 mg/kg DM) its use in the sheep and goat diets is limited (maximum 30% of DM) due to the relative sensitivity of sheep and goats to the chronic Cu toxicity. However the sheep/goat is the main and expanding Malaysian ruminant production industry and potential user of PKC if the bioavailability of the Cu in PKC could be reduced to prevent the problematic chronic Cu toxicity. The already known dietary Cu-binding supplements of Mo, Mo+S and Zn (2) were used in the present experiment with lambs and their Cu binding efficacy was compared by measurements of the relative Cu body retention, and of blood plasma and liver concentrations.

Twelve male 8-month old lambs were used in a 6-month feeding experiment to determine the effects of dietary Mo, Mo+S and Zn supplements on the body retention and liver accumulation of dietary Cu. The lambs were divided into four groups of three and each group was fed *ad libitum* one of four diets. A control diet was based (DM basis) on PKC (86.1%) and grass hay (10%)*;* it containd 15.1% crude protein, 44.2% acid detergent fiber and 22.9 µ/g Cu. Three additional diets were the control supplemented either with Mo (20 mg/kg DM), Mo (20 mg/kg DM) + S (1g/kg DM) or Zn (500 mg/kg DM). The lambs had a free access to deionised drinking water. At three months of the experiment, feces and urine were collected and sampled for six days. At the end of the experiment (6 months) blood was sampled and then the sheep were slaughtered. The liver was removed and sampled for chemical analysis.

Table 1. Excretion, retention, and concentration of Cu in blood plasma and liver of lambs

Item	Diet							
	Control		+ Mo		+ Mo+S		+ Zn	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intake (mg)	13.40	1.18	15.72	1.74	14.39	2.15	13.44	2.08
Fecal excretion (mg)	9.88[a]	1.02	14.39[b]	1.60	13.39[b]	2.17	12.36[ab]	1.74
Urinary excretion (mg)	0.22[a]	0.04	0.27[a]	0.12	0.64[b]	0.06	0.21[a]	0.05
Retention (mg)	3.30[a]	0.23	1.06[b]	0.11	0.36[a]	0.08	0.87[b]	0.31
Retention (%)	24.6[c]	1.0	6.7[b]	0.9	2.5[a]	0.7	6.5[b]	1.2
Blood plasma (µg/ml)	1.86[b]	0.07	1.81[b]	0.15	1.59[ab]	0.31	1.29[a]	0.23
Liver (µg/g DM)	1196[c]	27	571[b]	188	199[a]	83	673[b]	18

[a-c]Means within the same row followed by the same superscript letter are not statistically different (P>0.05).

In comparison with the control, each dietary supplement decreased ($P<0.05$) the Cu concentration in the liver, but only the Mo+S supplement decreased it to below 350 µg/g DM (Table 1). This was accompanied with the body retention of dietary Cu of 24.6%, 6.7%, 2.5% and 6.5% for the control, Mo, Mo+S and Zn treatments, respectively. The blood plasma concentration of Cu was decreased ($P<0.05$) by the Zn supplement, but was not affected ($P>0.05$) by the other supplements.

It was concluded that from the supplements tested only Mo+S appeared to be effective in reducing the retention and liver accumulation of the dietary Cu to safe level; the liver Cu concentration range of 350 to 1000 µg/g DM is generally indicative of the liver Cu overload and marginal toxicity (3). Therefore dietary Mo+S supplement appears to be effective in preventing chronic Cu toxicity in sheep fed PKC-based diets. Indeed a follow-up experiment shoved that the supplement in the amount of 8 mg Mo and 1 g S per kg of the PKC DM used in the diet of sheep is optimal for reducing the hepatic Cu concentration to a safe level, without increase in the hepatic concentration of Mo (1). Therefore, it can be concluded that PKC can be included in the diets of sheep and goats in any proportion if the appropriate amounts of Mo and S are mixed in the PKC portion, before it is used for the dietary inclusion.

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WHICH WAY TO GO IN ANIMAL WASTE MANAGEMENT?

H.K. Ong
Strategic Resources Research Centre, Malaysian Agricultural Research and Development Institute, Serdang, Selangor.
E-mail: keng@mardi.gov.my

There are many technologies on animal waste management. However, there has been some confusion among livestock producers on the choice that should be employed in their farms. Some have complained about the effectiveness of so-called effective microorganisms that they used, while others lamented on the unsuitable technology. This paper aims to return to the basics and gives a refresher to the producers on what they are going into when investing in animal waste treatment technologies.

There are normally various unit operations in any waste management technology. For example, anaerobic digestion that produces biogas is a unit operation, while solid separation that separates the solids from the slurry is another. Heaping up waste in windrows is a unit operation and aeration in an oxidation pond is another. Understanding the function of each unit operation in the whole waste management work flow will minimise error and maintain efficiency.

Basically, there are two main methods of biological waste treatment, namely aerobic and anaerobic methods. Aerobic treatment makes use of aerobic bacteria that requires oxygen, while anaerobic treatment makes use of anaerobic bacteria that cannot survive in the presence of oxygen. Composting is an example of aerobic process, whereby oxygen is provided naturally by frequent turning or by aeration. Biogas production by anaerobic digestion is an example of anaerobic treatment where biogas could be captured for use as renewable energy. Supplementing anaerobic digestion with a consortium of aerobic bacteria will not help since the aerobic bacteria will die in the absence of oxygen. Similarly supplementing composting manure with anaerobic bacteria will be futile since the anaerobic bacteria die in the presence of oxygen.

Within each biological method, there are various modifications and configurations with different degree of sophistications. For example, activated sludge process, in-vessel composter with aeration, and sequencing batch reactor process are all different configurations of aerobic process. On the other hand, attached growth process, fixed film, and upflow anaerobic sludge blanket are all different configurations of anaerobic treatment aimed at increasing efficiency. As well, a treatment technology can make use of both methods. For example, a primary waste treatment method can be anaerobic digestion with biogas capture, which would reduce BOD concentration of slurry by 90%. If the BOD of raw slurry is 5000 mg/L, the treatment slurry would still have 500 mg/L of BOD, which could subsequently treated by aerobic process.

In both aerobic and anaerobic treatment methods, sufficient retention time is required. Retention time in aerobic system is shorter (hours or days) than that of anaerobic system (weeks). Wash-out, short-circuiting and stagnant zones must be avoided, particularly if microbes are supplemented. In addition, microbes require acclimatization.

One misconception about waste treatment technology is that it can make the waste disappear altogether. In treating the waste, we are only transforming it from one form to another, making it more hygienic for further utilization. Therefore resource recovery is a logical target. For example, anaerobic digestion produces biogas which can be captured for use as renewable energy. The energy is mainly methane which

chemically consists of carbon and hydrogen only. Most of the other elements still remain in the sludge, which is a potential fertilizer either in the liquid or solid form. Therefore, one would need other unit operations to recover the sludge or it would accumulate in the tank or retention basin thus reducing its effective volume. If the solids are not recovered for utilization, then the recycling loop could not be completed thus making disposal a necessity. Unfortunately, "disposal" would never be a green solution since it is just passing the problem to some other localities or people.

On the above basis, the following is the suggested primary waste-treatment methods that can be adopted depending on housing design and management practices:
(a) If the waste is handled as manure together with bedding materials, it is suitable for composting. A case in point is that of broiler production, where batches of manure mixed with sawdust or wood shaving could be composted. The case is similar with layer production, where the manure can be periodically removed for composting. Pig farmers who produce weaners on deep litter could also employ this method since composting has already started on the deep litter itself. The final product is a fertilizer or at least a soil conditioner. (b) If the existing housing design allows for collection of manure either mechanically or by manual scrapping, composting is recommended since a saleable by-product is obtainable. Some feedlots and dairy farm may fall into this category. Again, the objective is to recover manure as fertilizers. (c) If the farmers have no choice, but to wash manure down with plenty of water as in piggeries and milking parlours, anaerobic digestion with methane capture is recommended since the slurry is amendable to biogas production. Several possibilities could be envisaged: i) In a small holder farm, a family-type digester could be built based on either Indian floating gasholder design or Chinese fixed dome design or modifications of both designs. Methane capture can be used for lighting and cooking, and sludge coming out in the effluent can be used to fertilizer home gardens. There is opportunity to practise integrated farming incorporating livestock and crops (could be food or feed) if land is available. ii) If the slurry is already being channelled into a series of lagoons, the anaerobic lagoon can be covered with HDPE sheet or red-mud plastic or other impervious materials to capture methane for powering a generator. The covered lagoon would act as an anaerobic digester and the power generated can be used for normal farm operations. In this case the farmer can consider upgrading the barns to closed houses with evaporative cooling to improve productivity since on-farm produced electricity is available. The digested solids or sludge could be removed either periodically or continuously using solids separators. These could be a boon or a bane. If utilized as fertilizer, they contribute to food or feed production. If disposed indiscriminately, they become a source of pollutants. iii) For large commercial farms with sufficient capacity, a purposefully designed biogas plant could be considered to take advantage of claiming carbon funds based on approved CDM (Clean Development Mechanism) methodologies of which bio-methanation is one. The additionality principle has to be adhered to, i.e. "the business as usual" was open lagoons system in which greenhouse gas was being continuously emitted. This case could also be considered by several or a group of farmers at the same localities, who amalgamate their farm-wastes and share a centralized biogas plant that is now sufficiently large to be considered by buyers of CER (certified emission reduction). A new PFA (Pig Farming Area) approved by a state government for pig production also falls into this category. The advantages are: 1) Recovery of renewable energy; 2) Recovery of fertilizers for feed production; 3) Creating opportunities for upgraded closed housing designs leading to improved productivity; 4) Economy of scale.

GROWTH PERFORMANCE OF COBB MALE BROILER FED ON RATION FORMULATED WITH 10% AND 20% CRUDE PROTEIN DERIVED FROM SPECIAL QUALITY PALM KERNEL MEAL AND PALM KERNEL EXPELLER

M.S. Farah Nurshahida[*], W.M. Wan Nooraida, M. Mardhati[1] and O. Atil[2*]

Energy & Protein Centre, MPOB Keratong Complex, Km 143, Lebuhraya Segamat Kuantan, 26900 Bandar Tun Razak, Pahang; [1]Livestock Strategic Research Centre, MARDI Headquaters, Serdang, Selangor; [2]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor.
*E-mail: os_atil@putra.upm.gov.my; farah@mpob.gov.my

The objective of this study was to investigate the feeding value of SQPKM as a potential source of crude protein for broiler starter and finisher. A growth trial was conducted on 810 day-old Cobb male broiler chicks. They were assigned to 5 treatments rations with 9 replications of 15 chicks per replication. They were subjected to group feeding in a cage of 1 m wide x 1 m long x 0.4 m high with starter and finisher rations. The chicks were fed with respective broiler starter rations from day one until day 21 and broiler finisher rations from day 22 until day 35. The rations were formulated on isocaloric and isonitrogenous basis. The crude protein (CP) of T1 was mainly from soybean meal. The CP of T2 and T3 came from special quality palm kernel meal (SQPKM) respectively, at 10 and 20% of the total CP content of the rations. In T4 and T5, 10 and 20% of the total CP content of the rations was contributed by the palm kernel expeller (PKE). Water was made available throughout the experimental period via nipple drinker *ad labitum*. The total amount of ration assigned to the group as per guideline from Cobb broiler nutrition schedule plus additional 5% (1).

Total feed consumed was recorded daily at 08:00 hours. Feed consumed was calculated by subtracting the feed left in the feed trough plus the feed spill from the amount added earlier. The chicks were fed with fresh rations daily. Growth performance data collected were total feed intake, total body weight gain and feed conversion ratio (FCR). The data were subjected to ANOVA. The difference existed among treatments were then analyzed using duncan multiple range test.

The body weight gain and total feed intake among treatments were significantly ($P<.05$) different during starter period (Table 1). Starter chicks that were consuming 10% CP from SQPKM tended to give higher body weight gain ($P<.05$) and consuming more palatable ration than T3 and T5. No different ($P>.05$) in feed intake among T2, T1 and T4 during the first 21 days of growth. The 10% total CP from SQPKM was effective to support good growth rate of Cobb male starter chicks similar to the reported value by the Cobb breeder guideline.

Total feed intake, total weight gain and FCR among treatments were significantly ($P<.05$) different during the 35 days growing period (Table 2). Male Cobb broiler birds fed on ration formulated with 10% total CP from SQPKM showed the best growth performance, highest in total body weight gain at 2.01 kg ($P<.05$) and the best FCR ($P<.05$) at 1.55 compared to T4 (1.90), T3 (1.99), T5(2.02), and T1(1.63, $P>.05$).

Table 1. Weight gain, feed intake and feed conversion ratio of male COBB broiler during 21 days of starter growing period.

Parameters	Treatments				
	T1	T2	T3	T4	T5
Average weight gain (kg)*	0.84 ± 0.03^{ab}	0.86 ± 0.01^{a}	0.65 ± 0.08^{c}	0.80 ± 0.01^{b}	0.50 ± 0.06^{d}
Average feed intake (kg)*	1.12 ± 0.04^{a}	1.15 ± 0.03^{a}	0.96 ± 0.07^{b}	1.13 ± 0.07^{a}	0.89 ± 0.09^{b}
FCR*	1.33 ± 0.07^{b}	1.34 ± 0.04^{b}	1.48 ± 0.22^{b}	1.41 ± 0.08^{b}	1.78 ± 0.19^{a}

*P<0.05; [a,b,c]Mean values with different superscripts within the same row is different (P<0.05)

Table 2. Weight gain, feed intake and feed conversion ratio of male COBB broiler during 35 days of growing period.

Parameters	Treatments				
	T1	T2	T3	T4	T5
Average weight gain (kg)*	1.98 ± 0.10^{a}	2.01 ± 0.09^{a}	1.39 ± 0.13^{c}	1.57 ± 0.09^{b}	1.11 ± 0.13^{d}
Average feed intake (kg)*	3.23 ± 0.22^{a}	3.12 ± 0.04^{ab}	2.77 ± 0.17^{c}	2.99 ± 0.07^{bc}	2.24 ± 0.39^{d}
FCR*	1.63 ± 0.08^{cd}	1.55 ± 0.08^{d}	1.99 ± 0.26^{b}	1.90 ± 0.10^{bc}	2.02 ± 0.50^{a}

*P<0.05; [a,b,c]Mean values with different superscripts within the same row is different (P<0.05)

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EFFECTS OF AN EXOGENOUS FIBROLYTIC ENZYME ON THE *IN VITRO* GAS PRODUCTION AND DRY MATTER DISAPPEARANCE OF OIL PALM FRONDS

M. Ebrahimi[1], M.A. Rajion[1*], Y. M. Goh[1], and A.Q. Sazili[2]
[1]Faculty of Veterinary Medicine, [2]Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
*Email: mohdali@vet.upm.edu.my

One feasible strategy for increasing livestock production is to utilize available indigenous feed resources. The oil palm fronds (OPF), which are made up of branches, stems and leaves, represent one of the most available and abundant agricultural by-products in Malaysia. We showed previously that the OPF contained high fibrous materials that cannot be completely digested by the ruminant (1). The application of fibrolytic enzymes to ruminant diets has increased recently due to enzyme-mediated increases in feed digestion *in vitro* (2). Fibrolytic enzymes for ruminants are used mainly based on their plant cell wall degradation capacity. The aim of this study was to evaluate the effect of adding an exogenous fibrolytic enzyme on the *in vitro* gas production and *in vitro* dry matter disappearance (IVDMD) of oil palm fronds.

Total gas production, gas production rate and IVDMD were evaluated using an *in vitro* gas production technique with rumen fluid as inoculums. The fluids taken from four rumen fistulated Kacang crossbred goats before morning feeding. The goats were kept in individually-housed pens and fed a commercial concentrate including 30% of oil palm fronds twice a day. Oil palm frond pellets were purchased from the Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. The OPF pellet and a standard commercial goat concentrate were ground to 1mm to be used in the incubation. A mixture of 0.25g of oven dried concentrate with no OPF (control), 0.20g of concentrate + 0.05g of OPF (OPF20) and 0.125g of concentrate + 0.10g of OPF (OPF40) were incubated, without or with addition of a cellulase enzyme (20 mg/kg DM) in a 100 ml calibrated syringe with 20 ml phosphate and bicarbonate buffer and 5 ml strained rumen fluid for 48 h at 39^{o} C (3). At the end of the trial total gas production, rate of gas production and IVDMD were measured. Data were analyzed by the PROC GLM of SAS (SAS Inst. Inc., Cary, NC) with class variables including different inclusion levels of OPF, enzyme and the interaction between OPF and enzyme. A two-way ANOVA procedure was used to test the main and interactive effects of the fibrolytic enzyme and different inclusion levels of OPF on *in vitro* gas production parameters and IVDMD. Significance between individual means was identified using the Tukey's multiply range test.

Gas production and IVDMD of the 48 h incubations are presented in Table 1. The cumulative volume (ml) of gas production increased with increasing time of incubation. Adding cellulase enzyme to 20% and 40% OPF gave a considerably higher gas production than that for OPF without cellulase enzyme. The addition of cellulase enzyme increased significantly the total and rate of constant gas production at the end of the 48 h incubation.

Table 1. *In vitro* gas production (ml/g DM), rate of gas production (ml/h) and IVDMD (%) of different inclusion levels of OPF in the presence or absence of cellulase enzyme after 48 h incubation

Diet	OPF Without enzyme			OPF With Enzyme			*P*-value			
IT(h)[a]	0	20	40	0	20	40	SEM	Diet	Enzyme	Diet * Enzyme
2	2.82	2.98	2.66	2.42	2.82	2.65	0.11	0.6256	0.4744	0.8326
4	5.33	4.68	4.36	4.60	4.86	4.35	0.19	0.5095	0.6747	0.6694
6	9.06	8.08	6.56	7.79	6.73	6.75	0.29	0.0750	0.1962	0.5245
8	12.11	10.28	8.60	11.16	9.28	9.55	0.35	0.0193	0.4609	0.6467
10	13.13	11.30	9.45	12.17	10.29	10.25	0.36	0.0122	0.5987	0.5217
12	14.65	12.83	11.14	13.69	11.82	12.62	0.35	0.0363	0.8216	0.2967
24	32.08	26.40	20.34	28.07	25.78	22.42	0.73	<.0001	0.3866	0.0490
48	40.20	33.53	26.28	40.02	35.56	30.79	0.87	<.0001	0.0227	0.1123
GPR[b]	0.84	0.71	0.55	0.83	0.74	0.64	0.02	<.0001	0.0331	0.1020
IVDMD[c]	60.67	48.13	44.48	61.16	52.16	47.58	2.18	<.0001	0.0421	0.0102

[a] IT: incubation time; [b] GPR: gas production rate; [c] IVDMD: i*n vitro* dry matter digestibility.

Increasing the levels of OPF decreased significantly (P<0.05) the disappearance of dry matter *in vitro*. Supplementation with cellulase enzyme showed a significant increase (P<0.05) of IVDMD at all inclusion levels of OPF compared to the treatment without cellulase enzyme. For example, the IVDMD with cellulase was improved (47.58%) in the treatment group with 40% OPF inclusion compared to treatment without cellulase (44.48%).

It can be concluded that an exogenous fibrolytic enzyme such as cellulase can increase the *in vitro* fibre degradation of OPF. Further studies with fibrolytic enzyme mixtures are required to determine the factors controlling the degradation of high fibre roughages.

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DETOXIFICATION OF *JATROPHA CURCAS* SEEDS THROUGH SOLID STATE FERMENTATION (SSF) BY USING *ASPERGILLUS NIGER*

A. Sharmila, A.R. Alimon*, M. Hilmi
Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400 Selangor
*Email: ralimon@agri.upm.edu.my

Jatropha curcas or known as *Jarak pagar* by Malaysian is a multipurpose plant that belongs to *Euphorbiacea* family. It contains several type of toxin; butphorbolester is the major toxic principles in *Jatropha curcas*. It is not vulnerable to heat, and it causes mortality to a number of animal species and human. The objective of this study was to investigate the effect of pre-treatment (soaking in 90% ethanol for 24 h) and solid state fermentation (SSF) using *Aspergillus niger* on the phorbolester concentration and chemical composition of Jatropha seeds. The inoculums for SSF were prepared until the spore suspension attained 1.0 x 10^6 spore mL^{-1}. The spores' number was estimated by direct microscopic counting using haemacytometer. Incubation period used for the fermentation of Jatropha was over the 7 days. Fermentation was carried out in the flask; where there are two types of substrates used; untreated and pre-treated (soaked in 90% ethanol for 24 hours) Jatropha. For each day, the flask was removed and samples of the fermented Jatropha were dried in oven at 60^{o}C for 48 hours. Samples were dried in forced draught oven to stop the fermentation, and were analysed for nutrients contents and phorbolester. Phorbolester was determined according to the method of Makkar and Becker (1). The result shows that there was no significant difference (P>0.05) in phorbolester concentration between untreated and ethanol pre-treatment, although there appeared to be a reduction of about 40% in the content of phorbolester in Jathropa when soaked in ethanol (Table 1).

Table 1. The effect of chemical pre-treatment followed by fungal fermentation on the proximate composition of *Jathropa curcas.*

	Effect of pre-treatment			Effect of fungal fermentation		
Nutrient (%)	Untreated	Ethanol treated	Sig	Untreated	Ethanol treated	Sig
DM	96.4±0.17	92.5±0.65		96.5±0.62	95.7±1.65	
Ash	5.0±0.01	4.57±0.06	NS	5.2±0.39	5.29±0.45	NS
CP	17.8±0.06	19.6±0.51	*	20.4±1.20	21.3±0.85	NS
EE	41.5±1.58	30.2±0.22	*	28.0±8.70	20.5±7.87	NS
ADF	40.9±3.49	35.5±0.42	*	29.9±4.52	32.9±2.04	*
NDF	57.4±3.96	54.5±3.28	*	45.2±6.98	48.3±4.48	NS
Phorbolester	4.9±0.06	2.72±1.35	*	1.92±1.41	1.54±0.88	NS

NS, not significantly different; *significantly different (p<0.05)

However, after 7 days SSF with *A. niger* the phorbolester was significantly reduced to less than 2.0 mg/g (Figure 1), but it was significantly difference (P<0.05) at different incubation time. At day 3, the phorbolester concentration for untreated and

pre-treated was highly reduced to about 60.61% and 51.1% respectively; however it tended to decrease in a small decrement progressively with time.

The chemical composition of untreated and pre-treated Jatropha was significantly different ($P<0.05$) between treatment and it was also significantly different ($P<0.05$) at different incubation time. CP content of pre-treated Jathropa was higher than untreated Jathropa with values of 20.35±1.2% and 21.29±0.85% respectively, but increased with increase time of fermentation. However, the ADF and NDF content of untreated (ADF; 29.93±4.52% and NDF; 45.15±6.98%) was lower than pre-treated (ADF; 32.94±2.04% and NDF; 48.31±4.48%), and they were continuously decreased over increased in incubation time for both treatment.

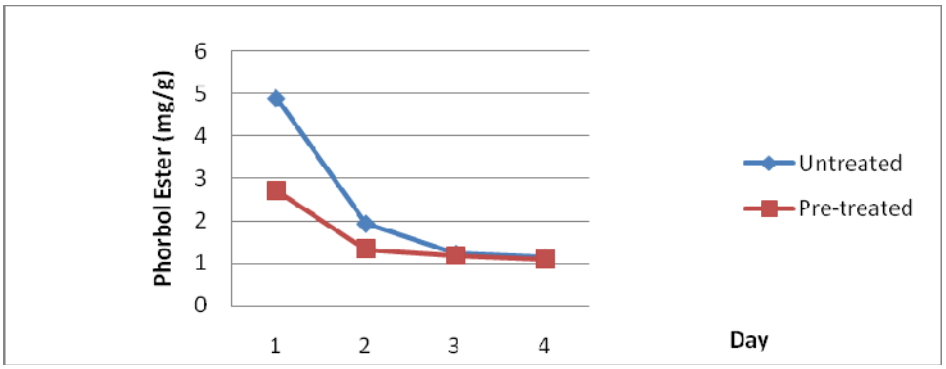


Figure 1. Phorbolester concentration of treated and pre-treated Jathropa seeds at 0, 3, 5 and 7 days fermentation with *A. niger*

It can be concluded that pre-treatment with 90% ethanol can lower the phorbolester content to some extent, while SSF using *Aspergillus niger* was successful in reducing further the phorbolester contents. A further advantage of SSF was that there appeared to be a small increase in the protein content possibly due to the reduction in fibre fraction or due to the presence of fungal biomass. Pre-treatment with ethanol before fermentation lower further the phorbolester content but not significantly different from that of the untreated samples.

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THE EFFECT OF DIETARY FLAVOURINGS (CINNAMON, ANISEED AND VANILLA) ON GROWTH PERFORMANCE OF BROILER CHICKENS

A.B. Nor Shafinas, A.R. Alimon* and M. Hilmi
Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
*Email: ralimon@agri.upm.edu.my

The flavour of feedstuff is an important factor that has long been considered in formulating animal rations (1, 2, 3). It has been thought that smell, taste and appearance of feed has an effect on intake and digestibility of feed through increased digestive enzyme production as a result of these senses. Studies on vanilla as a flavouring agent has been conducted by many researchers on cattle and sheep (4). However, the results are variable and not consistent especially in poultry as it was claimed that poultry do not have taste senses (3). In this study three types of flavourings are used to determine their effects growth performances and on carcass composition. In a completely randomized design experiment (4 treatments, 4 replicates and 10 birds per replicate), 160 male Cobb broilers chicks (14 days old, liveweight340-353g) were divided into four groups and given diets containing 0.5% aniseed powder, *Pimpinellaanisum L*. (Diet 2), 0.5% cinnamon powder, *Cinnamomumzylenicum,* (Diet 3), and 0.2% vanilla extract, *Vanilla planifolia,* (Diet 4) and 0% flavour (Diet 1; Control).

Table 1. Growth performance of broilers (mean ± SE) fed different food flavourings

	Diet 1	Diet 2	**Diet 3**	Diet 4
Initial body weight (g)	340±6.43^{a}	353±6.55^{a}	349 ±5.42^{a}	338±4.69^{a}
Final body weight (g)	2140 ±5.56^{b}	2364±10.11^{a}	2220±7.97^{b}	2141 ±10.4^{b}
Body weight gain (g)	1732±10.10 ^{b}	1967 ±9.2^{a}	1878±8.85^{ab}	1800 ±8.97^{b}
Feed intake (g)	3430 ±12^{a}	3486 ±17^{a}	3246 ± 12^{a}	3338±15^{a}
FCR (feed/gain)	2.13 ±0.09^{a}	1.64 ±0.06^{c}	1.76 ±0.05^{bc}	1.86±0.04^{b}
Digestibility DM (%)	78.7±1.67^{a}	79.9±1.78^{a}	80.97±0.10^{a}	78.79±0.44 ^{a}
Protein digestibility (%)	54.3^{a}±1.39	55.4±0.35^{a}	58.6±2.33^{a}	57.4±2.14 ^{a}
ME (MJ/kg)	12.5^{a}±0.16	12.5±0.31^{a}	12.9±0.07^{a}	12.5±0.09^{a}

Diet 1: without feed flavorings; Diet 2: 0.005% aniseed; Diet 3: 0.005% cinnamon; Diet 4: 0.002% vanillas.

The control Diet (Diet 1) did not contain any flavouring. The feeding trial was conducted until they were 42 days of age after which they were slaughtered and carcass characteristics measured. Feed intake, body weight and FCR were measured on a weekly basis. Table 1 shows the growth performance of birds fed different treatments. There were significant differences ($p<0.05$) in feed intakes, final body weights, body weight gains and FCR. The trend was also similar fordigestibility of dry matter (DMD), crude protein (DCP) and metabolizable energy (ME), where almost all treatments show significant increment when compared to the control group. Birds fed Diet 3 showed the highest DMD (80.97±0.10), DCP (58.64±2.33) and ME (12.91±0.07). The carcass composition was not significantly ($P>0.05$) different

between the treatments (Table 2). Of the flavourings used, aniseed appeared to significantly increased body weight and improved FCR compared to the control. The carcass composition in terms of meat, muscle and bone were not significantly different (P>0.05) among the treatments.

In conclusion, the results showed that the addition of flavourings in diets appeared to have a positive effect on growth performance and nutrient digestibility of broilers.

Table 2. Dissectible components of broiler carcass (mean ± SE) fed different food flavourings

	FAT (g)	BONE (g)	MUSCLE (g)
Diet 1	36.2 ± 5.86	159.3 ± 17.39	363.0 ± 30.41
Diet 2	28.9 ± 3.95	145.2 ± 13.86	420.9 ± 32.25
Diet 3	26.6 ± 3.63	151.8 ± 12.21	419.7 ± 12.74
Diet 4	34.8 ± 6.17	150.4 ± 3.80	398.6 ± 36.69

[a, b, c,] values within column with different superscripts are significantly different ($p<0.05$). Diet 1: without feed flavorings; Diet 2: 0.005% aniseed; Diet 3: 0.005% cinnamon; Diet 4: 0.002% vanillas.

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EFFECT OF COMMERCIAL FEED'S MOISTURE CONTENT ON PRODUCTION OF *MEGASELIA SCALARIS* (PUPAE PRODUCTION, WEIGHT AND ADULT EMERGENCE)

E.H. Tan[1*], B.H. Teo[1], A. Hamdan[1] and M. I. Kamarudin[2]

[1]School Of Biological Sciences, Universiti Sains Malaysia, Penang; [2]Livestock Technology Resources Development Division, Department of Veterinary Services Malaysia, Ministry of Agriculture and Agro based Industries, Wisma Tani, Blok Podium, Lot 4G1, Precint 4, 62630 Putrajaya.

[*]Email: friendlynut@gmail.com

Edible bird nest farming has become an important industry since years ago because it's medicinal benefits to human beings (1). There are few species of swiftlet (Ave: Apodidae) which can produce edible bird nest, such as *Aerodramus fuciphagus. Megaselia scalaris*, insect in the family Phoridae is an important food sources for swiftlet due to its small size and high versatility. Thus, mass production of this insect is important to support the feed sources of swiftlets in captivity.

However, the food sources or rearing medium to produce *M. scalaris* in laboratory is not well studied. Insectivorous in captivity requires balanced nutrient level diet for good development. Therefore, insects produced in laboratory must contain high protein and others nutritional value (2). To produce high quality insect, high protein insect diet must be utilized during rearing process. Most of high quality commercial feed for insect production are very costly. Hence, optimising used of these commercial feeds could save the production cost without compromising the qualities of insect produced (3).

Different moist concentration of insect feed can influence various stages development of *M. scalaris* (4). Thus, this experiment was conducted to develop an optimum moisture content of feed for mass production of *M. scalaris.* The percentage of pupae recovery, pupal weight and adult emergence are expected to be higher in the feed with moisture content in the range 50% - 70%.

A raw commercial broiler chicken feed was mixed with 40°C water in a ratio of 1:1 (50% water-Feed A), 1:5 (83.33% water-Feed B) and 1:7 (87.5% water-Feed C) respectively. The raw feed was mixed with water and stirred with a spatula until a mixture was formed. A total of 15.0g of feed mixture was weighed and put into a round plastic container (6cm x 4cm). One centimetre of diameter hole is cut in the center of the lids and closed with muslin cloth to allow air ventilation. Twenty first instar larvae of *M. scalaris* were introduced to the individual container and closed tightly. The container was then placed at room temperature and 70-80% relative humidity. Larvae development was observed daily until pupariation. The number of pupae recovered was recorded. Then, they were weighed by using electronic balance and the mean weight obtained was recorded. Pupae were left in the round plastic container until adults emergence. The number of adults that emerged was recorded. Five replicates were done for each moisture experiment.

This experiment was carried out to compare and determine the optimum moisture content of commercial broiler chicken feed on the mass production of *M. scalaris*. There was highest percentage of pupal recovery shown in feed A, followed by feed B. Both of them were not significantly different ($F=23.616$, $df=2,12$ $P>0.05$) from each other but were significantly different ($F=23.616$; $df=2,12$; $P<0.05$) from feed C. The highest weight of pupae can be obtained from feed A which present significant difference ($F=13.825$; $df=2,12$; $P<0.05$) from that of feed C but did not show significant difference ($F=13.825$; $df=2,12$; $P>0.05$) from that of feed B. It seems that

the larvae of *M. scalaris* can perform well in the feed with moisture content within 50-80%. The maximal mortality observed in feed C might contributed by the excessive water content in the feed. Larvae were flooded (Fig. 3) instead of moving freely on the feed as seen on feed A and B (Fig. 1 & 2). Thus, larvae in feed A and B were able to access and ingest the feed easily, resulting in higher weight (Fig. 1 & 2). Result also showed that the highest percentage of adult emergence was found in feed A, followed by feed B and C. However, they were not significantly different ($F=1.242$; $df=2.12$; $P>0.05$) from each other. This result showed that *M. scalaris* were able to survive on these three different moistures content feeds.

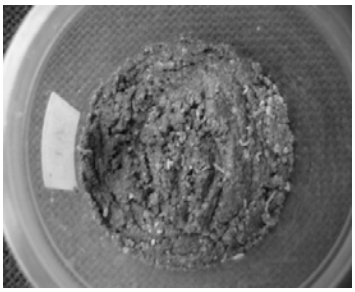


Figure 1: Feed A

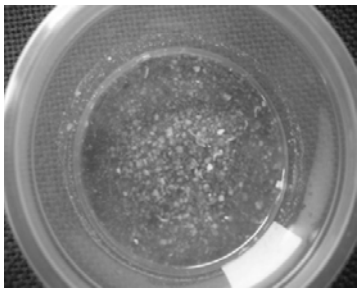


Figure 2: Feed B

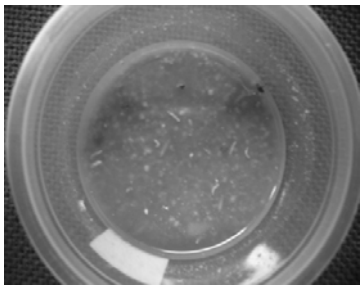


Figure 3: Feed C

From this experiment, commercial feed with 50-80% moisture content showed the best results in *M. scalaris* pupal recovery, pupal weight and adult emergence. Commercial feed can be mixed with water in the percentage range mentioned for mass producing *M. scalaris*. However, further study on smaller range of moisture content can be conducted to further evaluate the performance of the feed.

The authors would like to thank Khoo Choon Kiat for providing kind suggestions. Also, thank Encik Selveraja and Encik Guna Segaran for assisting us in the laboratory.

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EFFECT OF TEMPERATURES ON VARIOUS STAGES OF *MEGASELIA SCALARIS* DEVELOPMENT

B.H. Teo[1*], E.H. Tan[1], A. Hamdan[1] and M. I. Kamarudin[2]

[1] School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang; [2]Livestock Technology Resources Development Division, Department of Veterinary Services Malaysia, Ministry of Agriculture and Agro based Industries, Wisma Tani, Blok Podium, Lot 4G1, Precint 4, 62630 Putrajaya.

*Email: b_sien@hotmail.com

Megaselia scalaris (Loew) (Diptera: Phoridae) is a small and cosmopolitan fly (1). It is also known as "scuttle fly" due to its walking pattern by having a period of pause between the short bursts (2). It is about two millimeter long and yellowish in colour with some dark markings on the abdomen.

Megaselia scalaris is chosen to be developed as one of the feeds for domesticated swiftlets. This is because it is a smaller-bodied diptera which has been proven to be a favourite feed for swiftlets (3). It also can be cultured easily in the laboratory (4). However, until now, there is no study regarding the mass production of *M. scalaris* in laboratory.

The objective of our study was to examine the effect of different temperatures on developmental time for various stages of *M. scalaris*. Results of this study will provide information on the suitable temperature for mass-rearing of *M. scalaris* in laboratory which will enable shortening of its life cycle. This will increase the production of *M. scalaris* in short period of time.

In this study, three different temperatures: 19˚C, 27˚C and 35˚C were tested. This study was carried out at 75% relative humidity in dark condition. Six newly formed larvae of *M. scalaris* were put in a plastic container (4 X 6 cm) with a 1 cm diameter hole on the lid which was covered with muslin cloth. Five replicates were carried out for each temperature. Larvae were examined every 24 hours. Pupae formed in the plastic containers were individually transferred into small vials. Pupae were examined every 24 hours. Emergence of adults was recorded daily. Survival rate of larvae and pupae (%), larval and pupal developmental time (mean ± SE) were recorded.

The results indicated that there were no significant differences ($F=0$; $df=2, 12$; $P>0.05$) among the three temperatures for the survival rate of *M. scalaris* larvae. This showed that all larvae at the different temperatures successfully changed to pupae. However, there were significantly differences ($F=34.92$; $df=2, 12$; $P<0.05$) for the survival rate of *M. scalaris* pupae at three different temperatures. Mean percentage survival of pupae at 27˚C was the highest, followed by those at 19 ˚C and 35 ˚C. Trumble and Pienkowski (5) conducted a research on development and survival of *M. scalaris* at three different temperatures (21˚C, 27˚C and 32˚C) and found that there was an increase in larval and pupal survival rate when the temperature was increased.

There were significant differences among the three temperatures for the larval developmental time ($F=39.24$; $df=2, 9$; $P<0.05$) and pupal developmental time ($F=33.18$; $df=2, 12$; $P<0.05$) of *M. scalaris*. The shortest larval developmental time of *M. scalaris* was 35 ˚C followed by 27˚C and 19˚C; while the shortest pupal developmental time of *M. scalaris* was 27 ˚C followed by 35 ˚C and 19˚C. A similar situation was found in a study by Trumble and Pienkowski (5). It showed that increasing the temperature increased the rate development of *M. scalaris* larvae and pupae.

From this study, 27˚C was the suitable temperature for mass production of *M. scalaris* as it had high survival rate and short developmental time for larvae and pupae of *M. scalaris*.

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A TRACTOR-MOUNTED SELF-LOADING IMPLEMENT FOR APPLYING CHICKEN MANURE IN TWIN-ROWS CROP CULTIVATION

M. Mohd Fazly*, A. Aris, A.R. Norahshekin and B. Saleh
Mechanization and Automation Research Center, MARDI, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia.
*Email: fazlym@mardi.gov.my

This report focuses on a research work involving the design, development, and evaluation of a prototype tractor implement to apply chicken manure or similar organic matter, on twin-rows planted annual crops, such as sweet potatoes, stevia and some vegetables. Chicken manure is usually added to sandy soils which lacks in organic matter to support economic crop cultivation. The amount of chicken manure added is high up to 30-46 t/ha for sand tailing (1). In view of this, the manual method of manure application is costly for large scale crop production. From the literature, the authors estimated that about 2-3 man-days are required to manually spread one tone of chicken manure on one hectare of row crop such as sweet potato and vegetables (2). Thus, manual application of chicken manure is costly and very labor demanding. Thus, mechanization is needed for this task.

The implement was designed for use with crops that are planted with twin-rows spacing of 1.5 m. The implement utilizes two augers to meter and deliver the chicken manure to the ground at the required application rate in terms of kg/m row length. The implement was provided with a mechanism to load the chicken manure from a heap on the ground into the hopper bin. No labor is required to operate the implement apart from the tractor driver.

The design concept and considerations were developed for the prototype implement. The prototype implement comprises the following major components; a self-emptying hopper bin to contain the chicken manure or organic matter to be applied to the plot of land, a mechanism to load the manure into the hopper bin from a heap on the ground, a chassis with an axle and a pair of wheels to support the implement, two augers and trough laid beneath the hopper bin to meter and deliver the manure at the required rate, a stirring mechanism in the hopper bin to ensure that the manure flows freely into the trough below it, power transmission systems to drive the auger, stirrer, and loading mechanism.

The layout and configuration of the implement is shown schematically in Figure 1 and the constructed implement is shown in Figure 2.

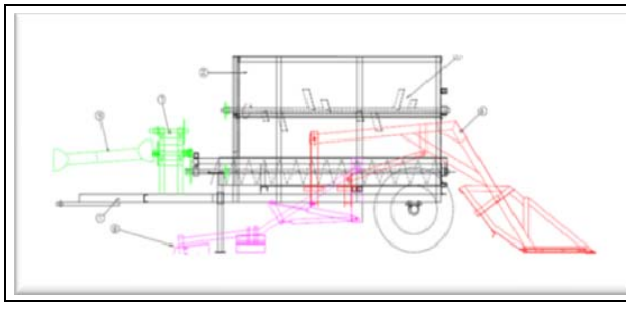


Figure 1: Schematic drawing of twin-rows



Figure 2: Constructed prototype organic manure spreader

The auger was chosen as the metering mechanism because of its simplicity, robustness and accuracy. The amount of manure metered out per unit time is directly dependent to its rotational speed. The auger spiral flight was constructed from 3 mm thick mild steel sheet. Each pitch length of the flight was made from a circular disc cut from the sheet. A central hole was drilled on each disc for the auger rod. The disc was then cut along one radius. The two cut edges were pulled apart in opposite directions and perpendicular to the face of the original disc to form the spiral of one pitch length. The complete auger flight was formed by welding several of the formed spirals on the auger rod. The outlet of the auger was positioned at the front end of the implement to enable the tractor driver to monitor the manure delivery.

The prototype implement satisfied almost all of its design requirements and performed as expected. Field capacities in the range of 0.18 ha/h to 0.30 ha/h were obtained. The plot performance tests were conducted on an actual sweet potato crop production plot of 0.2 ha in size on sand-tailings at MARDI Station, Kundang and bris soil at MARDI Station, Telong. The main observations made were on the effective field capacity, field efficiency, labor use, and durability under field conditions. Processed and raw chicken manure were used as the organic matter source. The cultural practice adopted for the crop involved depositing the manure inside a furrow dug in the ground on the planting rows. The furrows were about 200 mm deep, 400 mm wide at the top, and spaced at 1.2 m apart. It was dug using a double furrow opener drawn by a tractor. After the manure was placed in the furrow, it was closed using an open-ended V-shaped plates mounted on an implement carrier drawn by a tractor.

The desired application rates specified in the design requirements were mostly achieved during the functional tests. The rates of application were assessed using a 1.0 m wide plastic sheet put across the application path. The manure collected on the sheet was weighed giving the application rate in kg/m run. The application rates obtained during the functional tests were in the range of 1.4 to 3.6 kg/m run. Almost all design requirements set out for the prototype implement have been met.

The self-loading one-row implement to apply chicken manure on row crops has been designed, developed, and evaluated. The prototype implement satisfies almost all of its design requirements especially in terms of general performance, effectiveness in executing the design tasks, and owning and operating costs. There are 'unproductive' times, mainly for hopper bin loading, tractor turning, and travelling at the headland. This could be reduced in practice by better management of field work methods to improve the field efficiency.

The authors wished to thank the management and staff of MARDI Stations at Kundang, Telong, and Serdang for their valuable assistance during the machine field tests. Special thanks to my mentor Ir. Ayob Sukra for his dedication and valuable contributions on the development of this implement forms a component.

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EFFECTS OF FEEDING DIFFERENT METABOLITE COMBINATIONS PRODUCED BY *LACTOBACILLUS PLANTARUM* STRAINS ON EGG PRODUCTION PERFORMANCE, FAECAL pH AND FAECAL MICROBIAL POPULATION IN LAYING HENS

D.W. Choe[1], T.C. Loh[1,2*], H.L. Foo[4,5], Q.S. Awis[1] and M. Hair-Bejo[3]

[1]Department of Animal Science, Faculty of Agriculture, [2]Institute of Tropical Agriculture, [3]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, [4]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Science, [5]Institute of Bioscience, University Putra Malaysia, 43400 UPM, Serdang, Malaysia

*E-mail: tclohupm@yahoo.co.uk

For many years, antibiotics have been used in modern livestock and poultry farms not only to prevent or treat disease but to increase the growth performance and utilization of feed which ultimately reduces the cost of farm production. However, intensive and non-therapeutic use of antibiotics in agriculture leads to the development of antibiotic resistance, particularly in gut bacteria, such as *Enterobacteriaceae* (1). These resistant bacteria may be of public health concern as their resistance genes may spread to other bacteria through conjugative plasmids which could limit treatment options and delays recovery (2). The most troubling aspect is that antibiotic resistance can increase with continued and widespread use of antibiotics to improve growth. Currently, alternatives to in feed antibiotics are now available to improve growth in livestock which range from chemical and biological products to various feed ingredients and alternative feeding strategies. The present study was designed to determine the effects of different metabolite combinations produced from six different strains of *L. plantarum* on the egg production, faecal pH and faecal microbial population in laying hens.

A total of 200, 23-week old Lohman Brown laying hens were randomly assigned to four numerically equal groups, with 50 replicates per treatment, 2 birds per replicate. The dietary treatments consisted of: (Control) basal diet without metabolites supplementation; (COM246) basal diet with 0.6% metabolite combination from TL1, RI11 & RG11; (COM345) basal diet with 0.6% metabolite combination from RS5, RI11 & RG14 and (COM456) basal diet with 0.6% metabolite combination from RI11, RG14 & RG11. The experiment was conducted for 12 weeks with an addition of a week feed adaptation. Feed intake was measured by subtracting the left-over feed from the quantity originally supplied to the laying hens. Eggs from individual cages were collected daily and weighed. The hen-day egg production and feed efficiency were calculated as rate of production per hen per day and feed intake/egg mass.

The faecal pH was measured using Mettler-Toledo pH meter with glass electrode (Mettle-Toledo., England) and the faecal microbial population was determined using the method as described by Foo *et al*., 2003. 10% (w/v) of fresh faecal samples were diluted in sterile peptone water and left at room temperature for an hour prior to ten-fold serial dilutions (v/v). Enumerations of LAB were carried out on MRS agar (Lactobacillus agar DE Man, ROGOSA and SHAPE) (Merck, KgaA, Darmstadt) and ENT were spread and enumerated on EMB-agar (Eosin-methylene-blue Lactose Sucrose agar (Merck, KgaA, Darmstadt). The number of colony forming units (CFU) was expressed as the base 10 logarithm of CFU (logCFU) per gram. Data were analyzed by one way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure by Statistical Analysis System (SAS, 1998). Duncan's Multiple Range

Test System was used to compare the significant difference between the treatments at *P <0.05*.

There was no significant difference (P>0.05) among the treatment groups on overall feed intake, egg weight, egg mass and feed conversion efficiency. However, significant higher (P<0.05) overall hen-day egg production was observed in all treatment groups supplemented with metabolite combination as compared with the control. Highest egg production was seen in COM456. There was no significant difference (P>0.05) in faecal pH among the treatment groups at the first 4 weeks of sampling. However, COM456 started to show significant reduction (P<0.05) in faecal pH compared to the other groups at 28 weeks of age onwards. COM456 and COM246 started to exhibit significant (P<0.05) effect in reducing faecal pH compared to the control group from 30 weeks of age onwards. There was no significant difference (*P>0.05*) in LAB count among the dietary treatments at 24 to 28 weeks of sampling. However, COM456 started to show significant (P<0.05) higher level of LAB counts from 30 weeks of age onwards. COM246 showed significant (P<0.05) increase in faecal LAB counts on 32 weeks of age onwards and followed by COM345 at 34 weeks of age. In contrast, significant reduction of faecal ENT (P<0.05) were observed in all groups supplemented with metabolite combinations from 30 weeks of age onwards. The metabolite combinations produced by L. plantarum contain inhibitory compounds such as bacteriocins and organic acid (3). The acidifying effect of the organic acids (acetate, butyrate and propionate) provides a better environment for LAB to grow and suppresses pathogenic bacteria in the intestinal system therefore increasing its population (4). In addition, metabolite produces by LAB consist of antimicrobial compounds which inhibit the survivability of ENT. The lowering of faecal pH could be due to the acidifying effect of organic acids which is present in the metabolite (5).

In conclusion, supplementation of 0.6% COM456 exhibited best effect to improve egg production performance, reduced the faecal pH and ENT and increased LAB population. This indicate that metabolite from locally isolated *L. plantarum* is a possible alternative for in feed-antibiotics in poultry production.

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NUCLEAR MATURATION OF IMMATURE BOVINE OOCYTES VITRIFIED USING DIFFERENT CRYODEVICES

H. Hajarian[1], H.Wahid[1*], Y. Rosnina[1], M. Daliri[3], M. Dashtizad[1], T. Mirzapour[1], N. Yimer[1], M.M. Bukar[1], M.I. Iswadi[2] and O. Abas Mazni[2]

[1]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia; [2]Agro-Biotechnology Institute (ABI), 43400, Serdang, Selangor DarulEhsan, Malaysia; [3]Department of Animal Reproduction, National Institute of Genetic Engineering and Biotechnology, 14965 Tehran, Iran

*Email: wahid@vet.upm.edu.my

One of the most important current fields of research in cryopreservation of reproductive cells (oocyte, spermatozoa) and organs (ovary) is vitrification, which may be interpreted as a practical, reliable and cost effective method compared to the conventional slow freezing method (1). Vitrification is affected by many factors such as cooling/warming rates, quality of oocytes, and temperature during the equilibration processes. Until now different cryodevices have been employed to achieve high cooling/warming rates and also to decrease the concentration of cryoprotectants, such as open pulled straw (OPS), cryoloop, cut standard straw, electron microscopy grids (EMG), and cryotop (2-4). Some of the mentioned cryodevices due to their shape, material and direct contact with liquid nitrogen, interestingly increase cooling rates and decrease the need for highly concentrated cryoprotectants.To find out the suitable cryodevice for vitrification of immature oocytes, three different cryodevices (OPS, EMG, and cryotop) were employed and outcomes in terms of polar body extrusion and nuclear maturation after warming and *in vitro* maturation for 24 hourswere compared.

A total of 253 bovine cumulus-oocyte-complexes (COCs) were used. After recovery of oocytes, they were washed twice in washing solution (WS; M-199 + 0.4% BSA) and twice in holding solution (HS; M-199 + 20% FCS + Gentamycin) and left there for 15 min. Subsequently, they were equilibrated in the first vitrification solution (VS1;HS + 7.5% DMSO+ 7.5% EG) for 10-12 minand then in the second vitrification solution (VS2; 15% DMSO+ 15% EG +0.5 M Sucrose) for 60 sec. Within 60 sec they were mounted on one of the cryodevices and directly plunged into liquid nitrogen for 10 days. For warming, the cryodevices were directly introduced to HS containing 1.0M sucrose for one min and then rehydrated through series of 0.50, 0.25, 0.15, and 0 M sucrose in HS for 3, 3, 5, and 5 min respectively. Immature oocytes in treatment groups were cultured *in vitro* for 24 h. Aceto-lacmoid stain was used for detection of nuclear maturity stage. Significant differences among treatments used in the experiment were revealed by one-way analysis of variance and followed by Duncan's new multiple range test for mean comparisons ($p<0.05$) using SAS software ver. 9.1 (SAS Institute Inc., Cary, N.C.).

Polar body assessment of denuded oocytes revealed that vitrification using cryotop device increased the percentage of oocytes with first extruded polar body ($p<0.05$; Table 1). Although the result for cryotop group was significantly higher compared to the OPS and EMG groups, it was significantly lower when compared to the control group ($p<0.05$). The result also indicated that the control group showed the least degenerated oocytes followed by cryotop, EMG and OPS groups. There was no significant difference in terms of the absence of polar body (PB-) between OPS, cryotop, and EMG groups ($p>0.05$), but control group represented the lowest percentage of PB- compared to other groups ($p<0.05$; Table 1).Vitrified/warmed

immature oocytes at MII stage in cryotop group (58 %) were significantly higher compared to EMG and OPS groups (46% and 35%, respectively). Oocytes in cryotop and EMG groups tended to pursue their maturation and had less GV stage oocytes in comparison with OPS group ($p<0.05$; Table 2). There was no significant difference in percentage of GVBD-MI between treatment groups. In comparison with the other groups, the least GV and GVBD-MI rates resulted from control group ($p<0.05$).

It can be concluded that cryodevices which result in higher cooling/warming rates such as cryotop would be a better choice for vitrification of immature bovine oocytes.

Table 1: Polar body rate of immature bovine oocytes vitrified by different cryodevices

Groups	OPS (n=49)	Cryotop (n=58)	EMG (n=77)	Control (n=69)
Polar body +	10 (26%)^{c}	24 (41%)^{b}	24 (32%)^{c}	48 (70%)^{a}
Polar body -	27 (50%)^{a}	26 (45%)^{a}	39 (52%)^{a}	20 (29%)^{b}
Degenerated	12 (24%)^{a}	8 (14%)^{b}	14 (18%)^{ab}	1 (1%)^{c}

Data were pooled from 4 replicates. ^{a,b,c} Values with different superscripts in the same row are significantly different (P<0.05; ANOVA and Duncan's test)

Table 2: Nuclear maturation of immature oocytes vitrified using different cryodevices

Nuclear stage	OPS (n=37)	Cryotop (n=50)	EMG (n=63)	Control (n=68)
GV	15 (41%)^{a}	8 (16%)^{c}	16 (25%)^{b}	4 (6%)^{d}
GVBD-MI	6 (16%)^{a}	10 (20%)^{a}	13 (20%)^{a}	2 (2.5%)^{b}
MII	13 (35%)^{d}	29 (58%)^{b}	29 (46%)^{c}	60 (88%)^{a}
Unclassified	3 (7%)	3 (5%)	5 (7%)	2 (2%)

Data were pooled from 4 replicates. ^{a,b,c} Values with different superscripts in the same row are significantly different (P<0.05; ANOVA and Duncan's test)

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PRODUCTION OF CLONED CAPRINE BLASTOCYSTS USING INTRA- AND INTERSPECIES SCNT APPROACH

P.J. Kwong, W.E. Wan Khadijah* and R.B. Abdullah
Animal Biotechnology-Embryo Laboratory, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
*Email: wkhadi@um.edu.my

The emerging of successful reports on producing various cloned animal such as bovine, ovine,caprine and porcine since 1997 has led to the interest of many researchers in applying nuclear transfer technology not only in basic research but also in biomedical, agriculture and wildlife conservation fields. Generally, somatic cell nuclear transfer (SCNT) can be carried out using two approaches namely the intraspecies SCNT (intraSCNT) and interspecies SCNT (interSCNT). The available reports up-to-date on the production of cloned caprine embryos and offspring is through the integration of intraSCNT approach (1), and there are no any significant report by other researcher available on the production of cloned caprine via interSCNT approach, specifically using bovine oocytes as recipient cytoplastexcept the attempt conducted by our laboratory (2).This interSCNT approach is indeed useful to enable production of cloned embryos in circumstances where scarce oocyte resources of the domestic goats arise. Today, interSCNT using bovine oocyte as recipient cytoplasm is not being integrated solely to mass propagate superior animal for commercialisation purposes. Indeed its importance and potentials are foreseen to facilitate the conservation effort of endangered animals like gaur and banteng (3, 4). Therefore, in this present study, bovine oocyte is being integrated as the recipient cytoplasm to produce cloned caprine embryos with the futuristic view that this interSCNTapproach can be applied not only to save extinct caprine species like the serow goat but also as a technology to revitalise the present caprine breeding industry. Furthermore, most of the reports on successful production of cloned kids available were accomplished by transferring 2- and 4-cell cloned caprine embryos into the surrogate mothers (5), and not many reported on the *in vitro* developmental (IVD) competency of cloned caprine embryos up to blastocyst stage.

Therefore, the main aim of this study was to elucidate the possibility of producing cloned caprine blastocyst in *in vitro* culture (IVC) system via somatic cell nuclear transfer (SCNT) technique in Malaysia, while the sub-objectives comprise of: (i) to evaluate the IVD competency of LOPU-derived caprine oocytes versus abattoir-derived caprine oocytes and (ii) to compare the efficacy of intraSCNT (caprine-caprine) versus interSCNT (caprine-bovine) approach in producing cloned caprine blastocyst. The source of bovine oocytes used in interSCNT (caprine-bovine) was obtained from abattoir. The manipulation technique of SCNT used in this study involvedenucleation via squeezing technique followed by injection of caprine ear fibroblast cell into the perivitelline space of the enucleated oocytes and finally fused with electrofusion machine before subjected to chemical activation treatment and IVC. The data obtained were analysed using SPSS programme, means were analysed using ANOVA and differences among means were determine using DMRT. Significance was determined at $P \leq 0.05$.

In this present study, the *in vitro* maturation (IVM) rate for caprine oocytes derived from LOPU was significantly higher (77.5±3.37) compared to abattoir-derived caprine oocytes (63.11±1.69), while the IVM rate of bovine oocytes did not differ

significantly (73.9±1.41) compared to the overall caprine IVM rate (71.74±2.80) using the same IVC system. The cleavage rate, blastocyst rate and hatched blastocyst rate (77.55±5.14, 17.99±5.54 and 11.96±4.13) obtained for cloned caprineintraSCNT using LOPU-derived oocytes, respectively, did not differ significantly compared to using abattoir-derived oocytes (76.6±2.13, 9.52±1.75 and 5.69±1.78). A similar scenario was observed when comparison on cleavage rate, blastocyst rate and hatched blastocyst rate were made between intraSCNT approach (77.17±3.12, 14.60±3.49 and 9.45±2.64) and interSCNT approach (79.16±1.64, 6.29±1.24 and 3.5±0.89), where all did not differ significantly.

In conclusion, the findings of this study prove that cloned caprine blastocyst could be produced via SCNT in Malaysia in which the caprine oocytes IVD competency post-SCNT was not compromised regardless of its resources from LOPU or abattoir and both intraSCNT and interSCNT approach possess more or less the same efficacy in producing cloned caprine blastocyst. With routine production of cloned caprine blastocyst via interSCNT using bovine oocytes as recipient cytoplast, it is hoped that in the near future, uterine embryo transfer could be integrated into caprine management practices for profitable and sustainable caprinecommercialisation particularly in Malaysia.

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SERUM CALCIUM AND PHOSPHORUS LEVELS AMONG CATTLE IN SABAH

O. Rafidah [1*], A. Punimin[1], M. Zamri-Saad[2] and C.W. Lee[1]
[1]Department of Veterinary Services and Animal Industry, Sabah, [2] Faculty of Veterinary Medicine, Universiti Putra Malaysia.
*Email: Rafidah.Othman@sabah.gov.my / o_rafidah@yahoo.com

Calcium (Ca) and phosphorus (P) are two most abundant mineral elements in animal body. P deficiency is predominantly a condition of grazing ruminants, whereas Ca deficiency is a problem of animals fed mostly on concentrates (1). Disturbances in blood concentrations of these minerals can cause reduced feed intake, poor rumen and intestine motilities, poor productivity, and increased susceptibility to metabolic and infectious diseases (2). The aims of the study were to determine Ca and P status and to calculate the serum Ca:P ratio in cattle. A total 1,160 serums samples were randomly collected from cattle owned by government (5 farms) and private (2 farms) for a period of 2 years (2007; 2008). The Ca and P level was determined using a commercial (Eagle Diagnostic™) diagnostic kit. The Ca and P levels were classified as Low, Normal or High by comparing the measured values to the normal ranges (3); the Ca:P ratio was calculated (ideal Ca:P ratio is 2:1). Data were statistically analysed using the SPSS program.

In 2007, three (60%) of the government farms (G1, G4 and G5) showed significantly ($p<0.05$) high percentage of cattle with low serum Ca level. However, in 2008, two (40%) of the government farms (G1 and G3) showed low serum Ca level. Only one (50%) of the private farms (P1) had high percentage of cattle with low serum Ca level (Table 1). The serum P (inorganic) level indicated that all farms had significantly ($p<0.05$) higher percentage of cattle with hypophosphatemia in both years (Table 2). The findings for Ca:P ratio showed a serious imbalance as all farms showed significantly ($p<0.05$) low percentage of cattle with normal Ca:P ratio in both years.

Table 1: Percentage of cattle that were categorized as Low, Normal or High serum Ca concentration.

Year	Farms	Serum Ca Level, % (n, mean ±SD)		
		Low (< 8.00 mg/dL)	Normal (8.00 – 11.22 mg/dL)	High (>11.22 mg/dL)
2007	G 1	77 (75, 7.01±0.69)	23 (22, 8.24±0.20)	0
	G 2	36 (34, 7.44±0.54)	63 (60, 8.91±0.64)	1 (1, 11.30±0.00)
	G 3	41 (43, 7.39±0.34)	58 (62, 8.92±0.69)	1 (1, 11.90±0.00)
	G 4	53 (139, 7.29±0.47)	45 (118, 8.39±0.82)	1 (3, 11.40±0.00)
	G 5	75 (21, 7.05±0.54)	25 (7, 8.07±0.62)	0
2008	G 1	60 (25, 7.00±0.41)	40 (17, 8.39±0.43)	0
	G 3	82 (51, 6.90±0.66)	18 (11, 8.48±0.43)	0
	G4	36 (122, 7.34±0.43)	64 (220, 8.83±0.69)	0 (1, 11.90±0.00)
	P 1	72 (31, 6.75± 0.47)	28 (12, 8.58 ±0.59)	0
	P 2	41 (7 0, 7.36±0.42)	58 (98, 9.05 ±0.76)	1 (1, 11.80±0.00)

A (3)

The incidence of clinical hypocalcaemia and financial losses associated with the disease can vary enormously depending on the severity (4). This study revealed that many farms with low Ca levels, hypocalcemia prevention has to be taken seriously (5). On the other hand, all farms involved in this study were with serious hypophosphatemia

status and P deficiency can result in reduced overall productivity in cattle (6). Only sufficient diet that provided and a proper serum levels of Ca:P ratio are able to ensure that the animal will remain healthy and had a normal growth patterns (7). This study found that only 9 to 32 % of cattle studied showed the normal serum level of Ca:P ratio. Problems with animal health or performance are frequently the result of interaction between one or more minerals in the diet and not due to deficiency or excess per se (8).

Table 2: Percentage of cattle that were categorized as Low, Normal or High serum P concentration.

Year	Farms	Serum P (inorganic) Level, % (n, mean ±SD)		
		Low (< 3.50 mg/dL)	Normal (3.50 – 7.00 mg/dL)[A]	High (>7.00 mg/dL)
2007	Farm G1	99 (96, 2.28±0.46)	1 (1, 3.50±0.00)	0
	Farm G2	97 (92, 2.32±0.51)	3 (3, 3.80±0.30)	0
	Farm G3	99 (105, 2.02±0.37)	1 (1, 3.50±0.00)	0
	Farm G4	100 (175, 2.07±0.37)	0	0
	Farm G5	100 (28, 1.95±0.44)	0	0
2008	Farm G1	100 (42, 2.23±0.44)	0	0
	Farm G3	97 (60, 2.50±0.43)	3 (2, 3.50±0.00)	0
	Farm G4	97 (333, 2.32±0.51)	3 (10, 3.68±0.19)	0
	Farm P1	100 (43, 2.48±0.46)	0	0
	Farm P2	99 (167, 2.67±0.35)	1 (2, 3.60±0.00)	0

[A] (3)

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ECONOMIC OF CULLING COWS INDEX ON DAIRY HERD IN IRAN

A. Chizari[1]*, M. Zainal Abidin[1] and A. L. Ismail[1]
Department of Agribusiness and Information Systems, Faculty of Agriculture,
University Putra Malaysia[1], Serdang, Selangor.
*Email: ali.chizari.upm@gmail.com

One of the largest segments of the livestock sector in Iran is the dairy industry. Iran produces about 10 million tonnes of milk and is rated 18th in the world with 1,895,865 head of dairy cows registered in 14,645 farms. Due to high investments and long period in returns strategic farm management in dairy herd is important in order to earn a good return from each of the dairy cattle. In determining the performance of dairy cows, dairy farmers exercise different management approaches to manage their farm. These include: 1) Minimizing costs to get high profit; 2) minimizing assets per production unit to decrease fixed costs; 3) marketing milk to receive the best milk price possible to increase revenue; and 4) increasing production to maximize revenue and profit (1).

Culling is the departure of cows from the herd and this could be due to the animal being slaughtered, sold, salvage or death (2). There are two kind of culling 1) voluntary culling 2) involuntary culling. In voluntary culling decision initiated by the herd owner or manager and involuntary culling decision initiated by animal. Results indicated that involuntary culling probabilities have a large impact on annualized net revenue. Reducing involuntary culling rates by 2.9% resulted in about $22 more net revenue per cow per year (3). Approximately 34% of all cows are culled each year. Many dairy producers say that they have little and limited choice when it comes to culling dairy cows that do not generate enough milk sales to cover their variable cost generally should be culled (4).

Most of the past studies focus on two indices i.e. (i) comparing between culling factors and (ii) ranking the cows based on value. The type of index is very common and normally dynamic programming is being used to determine the cull index using the culling factors (5). The second index is basically based on the value of the cow itself using net present value and benefit-cost analysis. The results show that milk production and pregnancy are the most important factors for keeping the animal and for culling (4).

The general objective this study is to create a culling dairy cow index by reviewing individual performance factors by computing the Net Present Value of the animal. The specific objectives were: (1) to investigate the factors that influences the performance and value of dairy cow, (2) to create a decision making tool in culling and replacement the dairy cows and (3) to sort the dairy cows based on value and expected performances.

Statistical analysis of performance of neural networks has in many instances been compared to statistical results, especially regression analysis. The advantage of neural networks compared to statistical methods lies in the fact that when using traditional statistical methods, one must commit to a certain structure regarding the form of the desired function (6). More often than not, it is difficult to decide what the appropriate structure is especially in non linear problems. With a neural network we do not have to specify the specific structure of the desired function. Neural networks formulate the problem of parameter estimation and model-specification simultaneously. The last fact by itself makes a neural network rather attractive for solving non-linear mapping problems (6). Artificial neural networks are mainly used in livestock management. In dairy cattle farming, for example, artificial neural networks can be

successfully used to predict milk production per 305-d lactation, methods of predicting milk yield in dairy cows, effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle (7).

The dairy cow culling index is the best economic balance between maximize profit and culling of poor performance cows. The result will indicate which cows to be culled or not to be cull in order to gain higher productivity and profit for the farm.

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THE EFFECT OF USING DIFFERENT TYPES OF PALM KERNEL CAKE AS FEED INGREDIENT IN BROILER DIETS

M. Mardhati*, H.K. Wong, S. Noraini, M. Noraziah and A.W. Arifen
Strategic Livestock Research Centre, MARDI Headquarters, Serdang, Selangor
*Email: mardhati@mardi.gov.my

A major by-product of the oil palm industry is the palm kernel meal (PKM) with a total production of 2,312,222 tonnes (1), of which 95.6% was exported with export revenue of RM496 million. They are two types of PKM and they are the mechanically pressed palm kernel expeller (PKE) and the solvent extracted palm kernel cake (PKC). Palm kernel expeller is obtained as a by-product from the extraction of palm kernel oil via the mechanical process, while PKC on the other hand, is a by-product from the extraction of palm kernel oil via solvent (hexane) extraction of the ground palm kernels. The main difference between the solvent extracted PKC and expeller pressed type is in the ether extract or oil content with around 0.5-3% and 8-12%, respectively. The objective of this paper was to evaluate the growth performance, feed efficiency and carcass quality of broilers fed diets with similar levels of PKE and PKC.

A total of 96 day-old chicks (male, COBB 500) were reared in 2-tier battery cages in an open house system with 6 birds per cage. Birds were assigned to four dietary treatments - T1 corn-soya based ration as a control ration; T2, ration formulated with 20% palm kernel expeller; T3 ration formulated with 20% commercial solvent extracted PKC and T4 ration formulated with laboratory extracted PKC. The starter and grower diets were formulated according to the nutrient recommendations of the Cobb breeder company. The compositions of the diets are shown in Table 1. There were 4 replicates per treatment, with six birds per replicate. Rations were formulated to be isocaloric and isonitrogenous. Starter rations (1-20 days) and grower rations (21-42 days) were provided in mash form. The calculated nutrient compositions of the experimental rations were shown in Table 2. Feed intake and weight of the broilers were measured on day 21 and 36 to assess gain and feed conversion efficiency. The average broiler weight, total feed consumption and feed conversion ratio (FCR) were calculated for each ration. Growth performance was evaluated on day 1 - 20 for the starter period and day 21 - 42 for the grower period. Data obtained on each parameter were subjected to analysis of variance using SAS Statistical Package 9.1 (2) and significance was based on a 0.05 probability level.

The growth performance of broilers during starter period is shown in Table 2. Replacement of the corn and soybean meal in the diets with PKC or expeller was not significantly ($P>0.05$) affect the total feed consumption of the starter broilers, although the numerical values did not favour PKC/ PKE. The birds fed with solvent extracted PKC (T3 and T4) containing diet had the average body weight gain (ABW) significantly lower. The FCR was significantly ($P<0.05$) higher as compared to corn-soya based ration and PKE based ration. The lowest body weight was in the T4 group and this group also had the lowest feed consumption. The corn-soya based ration (T1) gave the best FCR compared to the PKC/PKE based rations. For the grower period (Table 2), the values for TFC were not significantly different ($p>0.05$) between treatments. The ABW for T1 was significantly higher ($p<0.05$) than the other

treatments. The FCR were significantly better ($p<0.05$) for T1 compared to T2, T3 and T4. Solvent extracted palm kernel based ration (T4) had the lowest ABW and highest FCR. The better growth performance and FCR for T1 can be attributed to better digestible nutrients in corn-soya based rations. Results from this study showed that the inclusion of PKC negatively affected the growth of broiler chicken.

Table 1. Proximate composition of experimental starter and grower rations for broilers.

Parameter	T1	T2	T3	T4
Starter ration				
Dry matter (%)	89.45	86.77	88.29	87.65
Crude protein (%)	22.20	21.09	21.42	21.91
Crude fiber (%)	1.35	3.59	3.55	2.58
Ether extract (%)	4.88	9.05	6.24	6.01
Ash (%)	6.86	5.51	6.06	5.71
Gross energy (MJ/kg)	16.78	17.87	17.22	17.04
Grower ration				
Dry matter (%)	85.99	85.21	86.64	86.83
Crude protein (%)	22.91	19.79	20.69	19.41
Ether extract (%)	4.00	9.19	5.24	8.44
Ash (%)	4.18	4.72	4.64	4.77
Gross energy (MJ/kg)	16.85	17.70	16.53	16.15

Table 2. Total feed consumption, average body weight and feed efficiency ratio of male COBB 308 broiler fed on T1, T2, T3, T4 and T5 (starter and grower period).

Parameter	T1	T2	T3	T4
Starter period				
TFC (kg)	$1.16^{a} \pm 0.03$	$1.15^{a} \pm 0.04$	$1.17^{a} \pm 0.02$	$1.13^{a} \pm 0.07$
ABW (kg)	$0.84^{a} \pm 0.03$	$0.82^{ab} \pm 0.02$	$0.79^{bc} \pm 0.02$	$0.75^{c} \pm 0.03$
FCR	$1.46^{b} \pm 0.06$	$1.49^{b} \pm 0.04$	$1.58^{a} \pm 0.07$	$1.61^{a} \pm 0.03$
Grower period				
TFC (kg)	$3.81^{a} \pm 0.13$	$3.94^{a} \pm 0.17$	$3.99^{a} \pm 0.35$	$3.88^{a} \pm 0.20$
ABW (g)	$2.28^{a} \pm 0.03$	$2.09^{b} \pm 0.10$	$2.08^{b} \pm 0.15$	$2.04^{b} \pm 0.11$
FCR	$1.67^{b} \pm 0.05$	$1.88^{a} \pm 0.02$	$1.92^{a} \pm 0.08$	$1.91^{a} \pm 0.05$

^{abc} Means in the same column with different superscripts are significantly different ($P<0.05$)
TFC – Total feed consumption; ABW – Average body weight; FCR – Feed conversion ratio

The inclusion of 20% PKE or PKC in broiler starter and grower diets negatively affected the growth of chickens compared to the corn-soya based diets. Therefore, there is a need therefore to carry out more research to improve the quality of the PKE and PKC in order to make it suitable to be used in broiler diets without affecting their growth performance, feed efficiency and production costs.

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EFFECTIVENESS OF PHYTASE PREMIX DEVELOPED FOR BROILER CHICKENS

Qurrata Aini[1, 3], K. Azhar [1*] and I. Aini [2]

[1]Dept. of Animal Science, Faculty of Agriculture; [2]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia; [3]Faculty of Animal Husbandry, University of Andalas, Padang, Indonesia

*Email: drazharkasim@yahoo.com

Phytase is an enzyme that improves phosphorus availability from phytate phosphorus as well as many other nutrients bound by phytate. It also has the potential impact to reduce environmental pollution by reducing the excretion of phosphorus in excreta (1). Microbial phytase is today widely used in diets for monogastric farm animals. Phytase enzymes may be included in poultry rations in the form of powder or liquid. For liquid enzyme products, a long shelf life is achieved by the addition of stabilizing agents (2). Inclusion of 1000 units per/kg feed of ASUIA279 phytase (centrifuged form) in the low P corn-soybean meal based broiler diet improved performance of broiler chickens (3). For farm level use a more practical form is the use of dry enzyme preparations. Different formulation technologies are used to produce enzyme powders that retain enzyme activity, stable in application, resistant to high temperatures, dust-free, and easy to handle. For the prevention of melting, the methods of drying, carriers are used, that can be organic material and inorganic material. Phytase enzyme is produced in liquid form. In this form, it is difficult to mix homogenous diets, not applicable at the farm level and it cannot be stable for long-term storage. Therefore, experiments were conducted to evaluate the potential of freeze dried phytase on performance of broiler chickens. There were five treatments: a control diet and four microbial phytase supplemented diets. The diets were formulated based on corn and soybean meal according to nutritional requirements (4) except for phosphorus. A total of 120 chickens were divided into five treatment groups, each group containing four replicates with eight chicks in each replicate pen arranged in a completely randomized design (CRD). The effectiveness of the microbial phytase on broiler performance over a period of 21 days was evaluated. Feed and water were made available at all times. Supplementation of freeze dried and liquid phytase significantly ($P<0.05$) improved body weight gain and FCR in comparison with the negative control diet (Table 1).

Table 1. Performance of broiler chickens (0-21 days) fed low phosphorus corn-soybean meal based diet supplemented with microbial phytase, ASUIA279 (1000units/kg feed).

Treatments	Weight gain (g/bird)	Feed intake (g/bird)	Feed conversion ratio
T1	701 ± 12^{bc}	1120±10	1.60 ± 0.01^{ba}
T2	677 ± 8^{c}	1142±21	1.69 ± 0.03^{a}
T3	699 ± 7^{bc}	1100±12	1.57 ± 0.02^{b}
T4	712 ± 7^{ab}	1120±5	1.57 ± 0.02^{b}
T5	744 ± 3^{a}	1125±7	1.51 ± 0.02^{b}

[a,b,c] Means in a column with different superscripts are significantly different at $P<0.05$ by LSD

T1=Positive control, T2=Negative control, T3=Negative control+powder phytase+rice hull, T4=Negative control+powder phytase, T5=Negative control+liquid phytase

Freeze dried phytase with rice hull did not give a positive response. For bone mineralization, only the liquid form gave positive effects on bone ash percent (Table 2). However, all the phytase supplemented diets significantly increased bone P content compared to the negative control diet.

Table 2. Phosphorus mineralization and mineral retention of broilers (0-21 days) fed low phosphorus corn-soybean meal-based diet supplemented with microbial phytase, ASUIA279 (1000units/kg feed)

Treatment	Bone ash	Bone phosphorus	Phosphorus retention	Phytate phosphorus retention	Calcium retention
T1	47.05±0.53^{ab}	15.09±0.97^{bc}	0.63±0.04^{a}	0.56±0.01^{ab}	0.64±0.01^{a}
T2	46.08±0.33^{b}	13.24±0.65^{c}	0.49±0.03^{b}	0.54±0.02^{b}	0.52±0.03^{b}
T3	46.29±0.48^{ab}	17.25±1.13^{ab}	0.50±0.03^{b}	0.55±0.03^{ab}	0.61±0.03^{ab}
T4	46.54±0.89^{ab}	19.09±0.28^{a}	0.54±0.04^{ab}	0.57±0.01^{ab}	0.65±0.01^{a}
T5	47.66±0.27^{a}	17.98±0.39^{ab}	0.63±0.01^{a}	0.61±0.01^{a}	0.61±0.02^{ab}

Means in a column with different superscripts are significantly different at P<0.05 by LSD
T1=Positive control, T2=Negative control, T3=Negative control+powder phytase+rice hull, T4=Negative control+powder phytase, T5=Negative control+liquid phytase

The liquid form of phytase improved both total and phytate P retention in comparison with the negative control diet. Supplementation of dried form of phytase numerically improved P retention compared to the negative control diet. However, the freeze dried phytase gave significant (P<0.05) improvement in Ca retention as compared to the negative control diet. The freeze dried phytase with rice hull did not improve the performance of broiler chickens in comparison with negative control diet. This might be due to the high fiber content of rice hull. The fiber fractions also negatively influenced the availability of minerals (5). Supplementation of freeze dried phytase is a potential alternative as an additive in broiler chicken feed.

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EFFECTS OF TWO FORMS OF PHYTASE EXTRACT ON PERFORMANCE OF BROILER CHICKENS

Khin San Mu[1, 4], K. Azhar [1*], I. Aini [2] and Che Roos Saad[3]

[1]Dept. of Animal Science, Faculty of Agriculture; [2]Faculty of Veterinary Medicine;
[3]Dept. of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor; [4]University of Veterinary Science, Yezin, Naypyi Taw, Myanmar
*Email: drazharkasim@yahoo.com

Supplementation of microbial phytase in soybean meal based broiler diets containing low phosphorus compensates for the adverse effect of low phosphorus levels in the diet by promoting improved bone mineralization and retention of nutrients such as Ca, P and CP (1). Agricultural residues are used for phytase production by solid state fermentation under submerged conditions, for enhanced phytase activity (2). In the report of (3), the addition of 5% rice bran in the LB **(**Luria-Bertani**)** media showed significant ($P < 0.05$) phytase production by all the bacterial strains tested. The highest activity was shown by ASUIA279 strain followed by ASUIA271 strain. Phytase used in the chicken trials was obtained from the liquid extract of the centrifuged LB rice bran media, referred to as 'centrifuged phytase extract'. Inclusion of 1000 units per/kg feed of ASUIA279 centrifuged phytase extract in low P corn-soybean meal based broiler diet improved the performance of broiler chickens (4). Therefore, it would be of great economic potential if the uncentrifuged LB rice bran media (containing phytase) can produce beneficial effects similar to the centrifuged phytase extract when included in chicken diet. The experimental diets were based on corn and soybean meal. Two basal diets containing 0.33% nPP (low P diet) and 0.45% nPP (recommended P, adequate diet) were formulated to meet nutrient requirements with similar levels of nitrogen, energy, calcium and other nutrients except non phytate P (nPP) content. Four replicate pens of 8 chicks were randomly assigned to one of four dietary treatments arranged in CRD for a study duration of three weeks. The experimental diets were 0.45% nPP (reference diet), 0.33 % nPP (low P diet), low P diet plus 1000 units/kg feed of centrifuged phytase extract and low P diet plus 1000 units/kg feed of uncentrifuged phytase extract. Both centrifuged and uncentrifuged phytase extracts supplemented to the P deficient corn-soybean meal P deficient diet gave significant improvement ($P<0.05$) in growth performance of broiler chickens (body weight gain and FCR) in comparison to the negative control diet (Table 1).

Table 1. The effect of centrifuged and uncentrifuged phytase extracts on growth performance of broiler chicken fed corn-soybean meal based low phosphorus diet at 21 days

Treatments	Weight gain (g/bird)	Feed intake (g/bird)	Feed conversion ratio
T1	701 ± 12^{bc}	1121±10	$1.60\pm.01^{b}$
T2	677 ± 8^{c}	1142±21	$1.69\pm.03^{a}$
T3	745 ± 3^{a}	1125±7	1.51 ± 0.02^{c}
T4	727 ± 15^{ab}	1121±6	$1.54\pm.04^{bc}$
P value	0.003	0.57	0.003

Means in each column with different superscripts are significantly different at $p<0.05$ by LSD; T1=Normal P, T2=Low P, T3=Centrifuged phytase extract, T4=Uncentrifuged phytase extract

The improvement made by centrifuged phytase extract supplementation was significantly better ($P<0.05$) than that of the reference diet (adequate P). Bone ash content and phosphorus retention were only significantly improved ($P<0.05$) by supplementation of centrifuged phytase extract and not influenced by uncentrifuged phytase extract supplementation. However, bone P content was improved by both extract supplementations to the low P diet (Table 2).

Table 2. Effect of centrifuged and uncentrifuged phytase extract on bone ash and P retention of broiler chicken fed corn soybean meal based diet

Treatments	Bone ash (%)	P in bone ash (g/100g)	Total P retention	Phytate P retention
T1	47.05 ± 0.53^{ab}	15.09 ± 0.97^{bc}	0.63 ± 0.03^{a}	0.56 ± 0.01^{b}
T2	46.07 ± 0.33^{b}	13.24 ± 0.64^{c}	0.49 ± 0.03^{b}	0.54 ± 0.02^{b}
T3	47.66 ± 0.27^{a}	17.98 ± 0.39^{a}	0.63 ± 0.01^{a}	0.61 ± 0.01^{a}
T4	46.70 ± 0.32^{ab}	$16.39\pm0.0.51^{ab}$	0.55 ± 0.01^{b}	0.56 ± 0.04^{b}
P value	0.05	0.002	0.01	0.002

Means in each column with different superscripts are significantly different at $p<0.05$ by LSD; T1=Normal P, T2=Low P, T3=Centrifuged phytase extract, T4=Uncentrifuged phytase extract

The response of broiler chickens fed centrifuged phytase extract supplemented low P diet was found to be better that that of broiler chickens fed uncentrifuged phytase extracts supplemented low P diet. However, growth performance was similar between the two treatments. It might be possible that the fiber and phytate of rice bran in the uncentrifuged phytase extract negatively influenced the availability of minerals (5). Based on the results on growth performance, uncentrifuged phytase extract could be utilized as an additive in broiler diets, thereby making centrifugation unnecessary. However, the nutrient matrix value of uncentrifuged phytase extract should be considered to get precise diet formulations.

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EFFECT OF FEEDING *CYMBOPOGON CITRATUS* LEAF AND *ZINGIBER OFFICINALE* RHIZOME ON GROWTH PERFORMANCE AND ILEAL LACTIC ACID CONCENTRATION IN BROILERS

K. Thayalini [1*], S. Shanmugavelu[1], P.M. Saminathan[2], M.S. Siti Masidayu[1], Y. Nor Idayusni[1], H. Zainuddin [1] and H.K. Wong[1]

[1]Strategic Livestock Research Centre, MARDI, P.O. Box 12301, 50774 Kuala Lumpur,
[2]Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Malaysia.
[*]Email: drlini@mardi.gov.my

Plants have been traditionally sourced as medicines for the treatment of various diseases. A number of dietary herbs, plant extracts and essential oils have been evaluated for their antimicrobial and growth promoter abilities in poultry (1). Some herbal supplements have improved growth performance, feed conversion efficiency, carcass and meat quality in broilers with, reduced feed cost (2). Lactic acid, the fermentation by product of *Lactobacillus* has demonstrated positive effect on the birds' performance (3). *Cymbopogon citratus* or lemon grass is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root (5). Both ethanol and aqueous extracts of *Cymbopogon citratus* leaf has shown to inhibit the growth of some gram-positive and gram-negative bacteria like *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus aureus* and *Escherichia coli*, during *in-vitro* studies (5). Ginger or *Zingiber officinale* on the other hand is a perennial herb with thick tuberous rhizomes (4). Ginger contains an enzyme called zingibain that aids digestion. Ginger extracts have shown to exhibit antibacterial activity under *in-vitro* studies (4). Therefore, a study was conducted to evaluate the inclusion of *Zingiber officinale* rhizome and *Cymbopogon citratus* leaf on growth performance and ileal lactic acid concentration of broilers. A total of 108- day-old male broiler chicks were randomly assigned to three treatment groups containing six replicate with six chicks each. The treatment groups were fed a corn soy-bean based diet supplemented with 2% dried *Zingiber officinale* rhizome and *Cymbopogon citratus* leaf respectively. The control group was fed a similar diet with no supplements. All the birds were raised in cages for a period of 42 days in cages with *ad libitum* assess to water and feed. Body weight and feed consumption were monitored at the beginning and at the end of study (42 days). At day 42, 1 g of ileal content was collected from one bird in each replicate into a test tube containing 1 ml of 24% meta-phosphoric acid in 2.5 mol sulphuric acid. The mixture was left overnight and the supernatant analyzed for non-VFA (lactic acid) concentration using a GC fitted with a flame ionization detector (FID), and nitrogen as carrier gas with a flow rate of 1.0 ml/min, after centrifugation. Data was analysed by SAS version 9.10.

During the first 3 weeks of the study, there were no significant ($P>0.05$) differences among dietary treatments in weight gain or feed conversion. However, body weight gain during the last 3 weeks and the overall period was significantly ($P<0.05$) different between the treatments with broilers fed *Cymbopogon citratus* leaf meal showing a significantly lower final body weight and body weight gain. The final body weight and body weight gain of the birds fed *Zingiber officinale* on the other hand were similar to those fed the control diet. There were no significant differences in feed conversion ratio among all treatments throughout the whole period. The lactic acid concentration was numerically higher with diets fed with *Zingiber officinale* although it was statistically similar with the control diet. In the present study, it can be concluded that supplementation with either *Zingiber officinale* or *Cymbopogon citratus* did not improve broiler performance despite higher lactic acid content with the

supplementation of *Zingiber officinale.* Furthermore, the performance of birds supplemented with *Cymbopogon citratus* leaf meal was inferior to the control birds.

Table 1: Growth performance and ileal lactic acid concentration of broilers fed herbal plants.

Parameters	*Zingiber officinale*	*Cymbopogon citratus* Leaf	Control	P-value
Body weight (g/bird)				
Day old	45.14±0.55	46.18±0.27	45.12±0.55	0.22
21d	761.43±13.71	743.37±20.99	769.28±24.73	0.66
42 d	2228.38±29.64[a]	2072.20±49.77[b]	2201.40±23.33[a]	0.02
Weight gain (g/bird)				
1 to 21 d	716.30±13.60	697.19±21.13	724.16±24.66	0.64
22 to 42 d	1466.95±36.35 [a]	1328.83±41.27 [b]	1432.12±14.62 [a]	0.02
1 to 42 d	2183.25±29.45[a]	2026.02±49.72[b]	2156.29±23.04[a]	0.02
Feed conversion ratio				
1 to 21 d	1.59±0.05	1.57±0.02	1.52±0.01	0.29
22 to 42 d	2.08±0.06	2.05±0.04	2.04±0.06	0.88
1 to 42 d	1.92±0.05	1.88±0.02	1.86±0.04	0.57
Lactic acid (µmole/g digesta)				
42d	70.62±17.00a[a]	19.85±5.17 [b]	37.19±16.43 [ab]	0.06

[ab] means (± standard error of mean) with different superscripts within the same row differ significantly (P<0.05)

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GAUGING THE SUPERIORITY OF MALES OVER FEMALES IN THE CROSSBRED KAMPUNG CHICKEN

M. Noraziah[1] and E.A. Engku Azahan[2*]

[1] Strategic Livestock Research Centre, MARDI, GPO Box 12301, 50774 Kuala Lumpur; [2]Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM, Serdang, Selangor

*Email: azahan@vet.upm.edu.my

In chickens, the superiority of males over females in terms of growth and feed conversion efficiency has generally been acknowledged (3, 4). This situation would inadvertently result in lower cost of producing male birds compared to females and hence can be sold at lower cost per unit body weight than females. Such price differentiation between sexes however is not practiced for commercial broilers in Malaysia. Male and female broilers are equally demanded and are sold at the same price per unit body weight.

Unlike the situation for broilers, kampung chickens are generally marketed according to sex. This is particularly true at wet market and farm levels. Female kampung birds are generally marketed at an average price of 18% higher than males (5). This can be due to their probable higher production cost as a consequence of their inferior feed utilization efficiency and growth compared to males, the basis for the quantum of the price differential is not known. There is no specific guideline for setting a price differential and it is not the same for all producers. Quantification of the superiority of males over females in relevant growth parameters could provide a guideline for the basis for setting a difference in market price between birds of the two sexes. This study was aimed at gauging this superiority.

One hundred and fifty crossbred kampung chickens from a popular strain were used in the study. They comprised of equal number of males and females. These birds were raised in individual cages from the ages of one day until 14 weeks. Commercial broiler feeds were provided *ad libitum* throughout the duration of the trial. Feed intake and body weight were recorded over the ages of 10, 12 and 14 weeks, which were the usual market ages for crossbred kampung chickens. Mortalities and when they occurred while feed conversion ratio was calculated for each bird. Differences in growth performance were analysed statistically and the superiority of one sex over the other, if any, was calculated.

Male kampung birds were observed to be superior to females ($p<0.01$) in all the parameters investigated over the three rearing ages studied (Table 1). The superiority of males over females generally increased with advancing age (Table 2). Superiority for body weight ranged between 26% and 33% while for feed conversion ratio (FCR), males were 10 - 13% superior to females. Male birds also consumed 14 - 17% more feed. Records of male superiority have also been observed in indigenous Nigerian chickens (1) and for commercial broilers (2, 4). For broilers, the degree of male superiority observed earlier (4) was numerically lower (20.5%) for body weight but higher for FCR (14.6%) than the figures obtained in the current study on kampung chickens.

Table1. Effect of sex on growth parameters of crossbred kampung chickens over rearing ages of 10, 12, and 14 weeks (means±std errors*)

Parameter	Age (weeks)	Male	Female
Body weight (g)	10	1747.7±22.3	1387.6±20.9
	12	2194.5±26.7	1689.0±25.0
	14	2603.2±29.9	1956.8±28.0
Feed intake (g)	10	3703.1±56.4	3259.6±52.8
	12	5113.2±72.3	4395.2±67.7
	14	6687.8±90.2	5736.6±84.5
Feed conversion ratio	10	2.17±0.02	2.42±0.02
	12	2.37± 0.02	2.67±0.02
	14	2.61±0.03	3.00±0.02

* at each rearing age, differences between sexes for all parameters investigated were significant ($p<0.05$)

Table 2: Superiority (%) of males over females in growth parameters of crossbred kampung chickens

Growth parameter	Market age		
	10 weeks	12 weeks	14 weeks
Body weight	26	30	33
Feed intake	14	16	17
Feed conversion ratio	10	11	13

Within the limits of the study conditions imposed, female birds need over two extra weeks of growing time to achieve body weights compared to those of males. Based on the FCR values observed, males were 10 - 13% superior to females. This would translate into a consumption of 10 - 13% less feed by males for a similar gain in body weight as compared to females. Therefore, based solely on feed consumed, females would cost 10 - 13% more than males to produce and consequently would have to be sold accordingly if they were marketed at the same age. The longer rearing period required by female birds to attain a particular targeted weight compared to males, with its associated extra labour, time and risk costs, is another factor that needs to be taken into account in the consideration of the differential in marketing price between males and females. Additionally, consumers' preference, if any, needs to be considered.

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EFFECTS OF FEED RESTRICTION ON CARCASS YIELD AND EDIBLE COMPONENTS OF CROSSBRED KAMPUNG CHICKENS

I.A. Azlina Azma[1] and E.A. Engku Azahan[2*].
[2]Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM, Serdang, Selangor[1]; Strategic Livestock Research Centre, MARDI, GPO Box 12301, 50774 Kuala Lumpur
[*] Email: azahan@vet.upm.edu.my

Purchasing of kampung chickens by Malaysians is mainly carried out by consideration of body weight of live birds, unlike the trading normally practiced for commercial broilers where eviscerated dressed carcasses and cut-up parts rather than fully feathered live birds are sold to the public. Consumers of kampung chickens need to be assured that the birds they are purchasing are real kampung chickens based mainly on plumage characteristics, and not spent layer hens or small size broilers; hence the necessity for the live sale of kampung chickens. Feeding systems practiced for kampung chickens vary widely, ranging from *ad libitum* feeding on complete feeds to various degrees of feed restrictions with or without supplementations. With such a varied nutritional regime, variations in live performance, and possibly carcass yield as well, would be expected to occur between feeding regimes. While consumers apparently may seem not to lose out on any variations in growth as a result of nutrition since body weight is the sole criterion in the purchase of kampung chickens, declines in percentage yield of the edible portions relative to body weight would represent a loss to the consumer; similar body-weight birds with higher percent carcass yield would obviously represent a better economic proposition than those with lower percent yield. This study attempted to examine variations, if any, in the yield of the edible portions of a popular strain of kampung chicken subjected to feeding restrictions.

Carcass yields of a strain of crossbred kampung chickens raised under three different feeding regimes were compiled and collated. The feeding regimes, comprising two restricted feeding schedules and an *ad libitum* feeding program, were as follows:

1. *Ad libitum* feeding - Three weeks on 22% crude protein (CP) starter feed and nine weeks on 19% CP finisher feed
2. Mild feed restriction – 80% of calculated *ad libitum* consumption for each of the above starter (3 weeks) and finisher (final 9 weeks) feeds
3. Severe feed restriction – 50% of calculated *ad libitum* consumption for each of the above starter (3 weeks) and finisher (final 9 weeks) feeds. These birds also received unknown amounts of supplementary nutrients from the surrounding.

The *ad libitum*-fed and the mild feed-restricted birds were fully confined in sheds while the severely feed-restricted birds were raised within a fenced-in open area with natural vegetation. All experimental birds were females and they were slaughtered at the age of 12 weeks. Data on dressed edible yield and the yields of the three most desirable portions (breast, thigh and drumstick) were statistically evaluated for differences between feeding regimes.

Under *ad libitum* feeding on commercial feed, some 72% of live weight was recovered as whole dressed eviscerated carcass (Table 1), similar to figures reported earlier (1, 2) for kampung chickens. On the other hand, birds on both feed-restricted regimes yielded significantly less ($p<0.05$) dressed carcass of under 60% of live weight, compared to the *ad libitum* fed chickens. Between the two restricted feeding regimes however, the difference in dressed yield was not significant.

Table 1. Yields of edible parts (as percentage of live weight) of crossbred kampung chickens reared under restricted or *ad libitum* feeding regimes (means ± std. deviation)

Feeding regime	Dressed yield	Breast	Thigh	Drumstick
Ad libitum (ad lib) -Starter 22% CP, Finisher 19% CP	71.9 ± 1.5[a]	19.5 ± 2.3[a]	11.1 ± 0.6[a]	10.7 ± 0.4[a]
Mild restriction (80% *ad lib*) - Starter 22%, Finisher 19%	58.5 ± 1.5[b]	15.3 ± 1.3[b]	9.5 ± 0.8[b]	9.0 ± 0.7[b]
Severe restriction (50% *ad lib*) - Starter 22%, Finisher 19%	57.0 ± 2.1[b]	15.5 ± 0.9[b]	9.5 ± 0.2[b]	9.3 ± 0.3[b]

[a, b] – within column, means with different letters differed ($p<0.05$)

The recorded yields for the breast, thigh and drumstick of the *ad libitum*-fed birds were 19.5, 11.1 and 10.7%, respectively. These mean values were statistically higher ($p<0.05$) than the values of the corresponding parts of birds from the two restricted feeding regimes. Differences in the three component parts between the two feed-restricted treatments were again small and not significant.

Superiority in yields of the *ad libitum*-fed over the feed-restricted birds ranged between 18.6 and 20.7% for whole dressed carcass while for breast, thigh and drumstick the mean superiority figures were 21.0, 14.4 and 14.5%, respectively. Feed restriction probably resulted in undersupply of protein which subsequently reduced muscle development. The probable lack of nutrients in the feed restricted birds could have resulted in the diversion of relatively more nutrients to non-edible parts such as feathers, legs and bones rather than to the edible components.

The failure of the two differing feed-restricted groups to show significant differences in carcass yields between themselves despite being offered differing amounts of commercial feed could probably be explained by the fact that the severely-restricted birds undoubtedly obtain some unquantifiable amount of nutrient supplements from the surrounding vegetation and environment.

It is concluded that restricted feeding regimes adversely affected the development of edible carcass parts in relation to overall growth of crossbred kampung chickens through insufficient supply of nutrients. Consumers purchasing kampung birds of similar body weights but reared on different feeding regimes therefore may not obtain similar amounts of either edible carcass or popular components.

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INTESTINAL CHANGES IN BROILER CHICKEN FED ON DIETS SUPPLEMENTED WITH *ANDROGRAPHIS PANICULATA* AND *ORTHOSIPHON STAMINEUS*

M. Malahubban,[1] A.R. Alimon,[1,2*] A. Q. Sazili[2] and S. Fakurazi [3]
[1]Institute of Tropical Agriculture, [2]Faculty of Agriculture, [3]Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
*Email: ralimon@agri.upm.edu.my

Utilization of supplements such as enzymes, probiotic, prebiotics and herbs are some of the nutritional strategies recommended to minimize the growth of pathogens and to improve performance of broilers fed diets without in-feed antibiotics. Improvement in growth performance (1), may be due to modifications of the digestive tract of poultry. It has been established that *Andrographis paniculata* (AP) increased live weight of broiler (2). Similarly, the leaves of *Orthosiphon stamineus* (OS) has been shown to exhibit pharmacological properties such as antioxidant, antibacterial, heptoprotective, antihypertensive, diuretic and anti-inflammatory (3). AP has been traditionally used as an anti-inflammatory, hepatoprotective, antiviral, antioxidant, immune enhancement herbal medicine (4). The objective of this study was to investigate the effect of different levels of AP and OS supplementation on the morphology of intestinal tract. In a CRD experiment, 280, 21-day old Cobb broiler chicks were divided into seven treatments groups, and fed basal diets containing 0, 0.2 % , 0.4 % or 0.8% AP or OS, respectively. The herbs were added in the form of powdered dried ground leaves. The basal diet was formulated according to National Research Council (NRC, 1994). At 42 days 10 birds from each group were selected, weighed and slaughtered. The small intestine (3 cm in length) was taken for histological measurements. Intestinal samples were analyzed according to the procedure of Goodlad et al (5). The length of ten villi and twenty crypts from each segment were measured using an optical microscope (Nikon). Magnification was set at 4x for villi and crypts. Data were analyzed by one-way analysis of variance. The intestinal weight and intestinal length of broiler supplemented with different level of AP and OS are shown in Table 1. The weight of small intestine of birds supplemented with 0.8% OS *were* significantly higher ($P<0.05$) than that of birds fed on Control diet. However, the length of intestinal with birds supplemented with 0.8% OS diet were not significantly different ($P>0.05$) compared to Control diet. The villus length in the duodenum (1749 µm) was significantly higher ($P<0.05$) in birds on diet 0.8% OS compared to birds fed the Control diet (1150 µm). However, crypt depth in duodenum (181±7.3 µm) was significantly lower ($P<0.05$) on birds supplemented with 0.8% OS as compared to Control diet on duodenum (258±8.1 µm). The treated birds showed shallower crypts and longer villi except broiler fed on diet supplemented with 0.4%AP, which is a satisfactory result when compared with Control. Length of villi is related to the digestive capacity and intestinal absorptive area. The higher microbial population leading to destruction and atrophy of villi and can cause shorter length of intestinal villi (6). Present study suggests that the antimicrobial properties of AP and OS played vital role in balancing the intestinal microbial population, resulted in much longer intestinal villi length in broiler fed on diet supplemented with respective herbs as compared to Control diet. According to Gabriel et al (7), the increased villus length and surface and then lower crypt depth showed an intestinal structure more oriented to digestion, with improved absorptive and hydrolysis potential than to nutrient requirement for intestinal maintenance. It can be concluded that diets supplemented with 0.8% OS resulted in

larger villus height, while the crypt depth was lower in the duodenum of broiler chicken.

Table 1: Mean (±S.E.) intestinal weight (g) and intestinal length (cm), villus length(µm) and crypt depth (µm) of broiler fed different supplemented with AP and OS at 42 d

Diet	Small Intestine			
	Weight (g)	Length (cm)	Villus length (µm)	Crypt depth (µm)
Control	109.4±3.29^{bc}	188.6±4.49^{ab}	1150±38^{d}	258±8.1^{a}
0.2%AP	84.0±5.05^{d}	190.0±3.94^{ab}	1227±48^{d}	180±5.9^{c}
0.4%AP	97.7±6.37^{cd}	178.1±8.82^{b}	1411±50^{b}	178±6.2^{c}
0.8%AP	117.4±8.28^{ab}	202.9±8.50^{a}	1242±45^{cd}	184±6.4^{c}
0.2%OS	115.4±4.32^{ab}	201.6±5.72^{a}	1470±55^{b}	218±7.4^{b}
0.4%OS	122.2±3.93^{ab}	205.6±7.83^{a}	1381±40^{bc}	227±7.9^{b}
0.8%OS	132.4±7.22^{a}	205.8±8.58^{a}	1749±69^{a}	181±7.3^{c}

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EFFECT OF LYSINE FROM FERMENTED POME ON BROILER PERFORMANCE

H. Jame'ah *, S. Noraini, A. R. Abd. Rahman, A. Ahmad, A.W. Norrizan and M.N. Noraishah

Strategic Livestock Research Centre, MARDI HQ, G.P.O Box 12301, 50774 Kuala Lumpur.
*E.mail: jameah@mardi.gov.my

Protein is a very important nutrient requirement for poultry feed. The most influential value of protein is amino acids content. The ideal protein and amino acid formulation in poultry feed would consist of highly digestible protein sources that complement each other, formulate the diets on digestible amino acid basis, and utilize commercially available free amino acids to compliment the requirements for methionine, methionine plus cystine, lysine, and threonine (1). Lysine is one of the most limiting amino acid in corn-soybean meal diets (2). Lysine is chosen as a reference amino acid and is utilized as the basis to evaluate the content of other essential amino acids balance in poultry diets (3). In a previous study, fermentation of palm oil mill effluent (POME) improved amino acids composition especially essential amino acid. In. broiler, essential amino acids digestibility of fermented POME was improved with lysine showing the highest percentage of improvement (4). With this availability, substitution of fermented POME in diets should give similar effect on broiler performance. In order to test the utilization of lysine from fermented POME in poultry diets, two different percentage of sample and commercial lysine were used for comparison. Thus, the objective of this study is to evaluate the effects of lysine from fermented POME on broiler performance.

In total, 200 day old broiler Cobb (female) were fed commercial starter diet at the beginning of the experimental period. On day eight, 10 birds of similar average body weight were selected and allotted to five treatments. Three replicates of each treatments with ten birds were divided into 15 groups (n=10) according to factorial arrangement based on a completely randomized design. Water and experimental diets were freely available and diets were formulated to meet the requirements for all essential nutrients with the exception of lysine. Experimental feed were based on a mixture of corn-soybean meal basal diets with graded levels of lysine (0, 0.1 and 0.2%) from L-Lysine HCl and fermented POME (5). Experiments were carried out until day 42, where birds and feed were weighed weekly for determination of weight gain, feed intake, and feed efficiency.

This study was conducted to prove the availability of lysine in fermented POME on broiler growth performance. The mean body weight, weight gain, feed intake and feed conversion ratio of broilers fed with graded levels of lysine after 42 days is presented in Table 1. The body weight and weight gain in female broilers shows normal increment with no significant difference. Even though feed conversion ratio of lysine from fermented POME is slightly different there is no significant difference. Other than lysine, fermented POME contains other amino and this may give the differences results for treatment D and E. Substitution of lysine from fermented POME in feed formulation need to consider the presence of other amino acids. The growth performance of broiler fed the lysine deficient basal diet supplemented with lysine from fermented POME and L-lysine HCl revealed no difference and this result was compatible to previous study by Emmert (5) which employed liquid lysine source in diet.

Table 1. Growth performance of broiler fed with different level of lysine

Diet type	Body weight (kg)	Weight gain	Feed intake (kg)	FCR
A. Basal Diet (BD)	1.77[a]	1.48[a]	2.67	1.80[a]
B. BD + 0.1% L-lysine.HCl	1.78[a]	1.49[a]	2.68	1.79[a]
C. BD + 0.2% L-lysine.HCl	1.76[a]	1.48[a]	2.58	1.75[a]
D. BD + 0.1% Fermented POME	1.72[a]	1.43[a]	2.73	1.91[ab]
E. BD + 0.2% Fermented POME	1.67[ab]	1.38[ab]	2.66	1.92[ab]

[a-b] Means within a column with different superscript differ significantly ($P < 0.05$)

There are no significant different on growth performance of broiler fed with fermented POME and commercial product. In conclusion lysine from fermented POME can be used to substitute commercial lysine (L-lysine. HCl). Further study on feed formulation with fermented POME could improve broiler growth performance.

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COMPARISON OF "KAMPUNG" AND COMMERCIAL CHICKEN EGG BASED EXTENDERS ON CRYOPRESERVED GOAT SPERM MOVEMENT CHARACTERISTICS

C.W.K. Janice[1], K.D.S. Kanwal[1*], W.E. Wan Khadijah[2] and R.B. Abdullah[2]
[1]School of Sustainable Agriculture, University Malaysia Sabah, Kota Kinabalu, Sabah; [2]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur
*Email: kkanwal@ums.edu.my

Techniques of cryopreservation resulted in loss of viable sperm during freezing-thawing process even though with significant improvement over the last several decades (1-3). The main sources of sperm cryoinjury are cold shock, osmotic stress, ice crystal formation and/or oxidative damage, which finally cause loss of sperm viability and fertility (4-6). High survival and fertility rates of bull sperm in extender with 20% egg yolk has been reported (7). Specifically chicken eggs yolk and its components can help in providing protection against cold shock (8). These protective effects are mainly attributed to the low-density lipoprotein (LDL) in the egg yolk (9-10). Semen extender containing yolk from eggs of avian species has been studied extensively using commercial chicken eggs (11-12).

Sperm cryopreservation in goats causes reduction in sperm motility which could adversely affect the fertility in artificial insemination (AI) programme. This study was conducted to evaluate the effect of yolks of different sources used as cryoprotectant on the goat sperm movement characteristics. Semen was collected from each buck 2 to 3 times weekly by using artificial vagina (AV). Semen was processed immediately after collection and was diluted with "kampung" egg yolk extender or commercial yolk extender. The diluted semen sample was frozen by using goat cryopreservation protocols established in University of Malaya, Malaysia. The frozen semen was stored in liquid nitrogen tank until further use. Fresh and frozen-thawed sperm movement characteristics were analysed for progressive motility (PM), rapid motility (RM), medium motility (MM), slow motility (SM), static, average path velocity (VAP), progressive velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN) using computer assisted semen analysis (CASA, Hamilton-Thorn, USA). Diluted fresh semen showed significantly higher sperm motility than frozen-thawed sperm in both "kampung" (FT-KEY) and commercial egg yolk (FT-CEY) extenders (94.79±1.06% vs. 77.27±2.93% and 71.71±5.61%, respectively); however, no significant difference was observed on sperm motility between FT-KEY and FT-CEY. Similar trends were also observed for other sperm movement characteristics.

In conclusion, although insignificant difference on frozen-thawed sperm movement characteristics between both extenders used in this study, apparently kampung chicken egg extender gives better cryoprotection effects compared to commercial egg extender during goat sperm cryopreservation. However, further refined research is needed to elucidate and improve "kampung" chicken egg extender so that it can be an alternative choice to other extenders in goat sperm freezing protocol for the industry in the future.

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THEPERFORMANCE AND NUTRIENTS DIGESTIBILITY OF BROILER CHICKEN AS AFFECTED BY DIETARY CRUDE PROTEIN, METHIONINE AND PUTRESCINE

S.M. Hashemi [1], T.C. Loh[1], H.L. Foo[2], **I. Zulkifli[1] and M. Hair-Bejo[3]**
[1]Animal Sci. Dept.; [2]Bioprocess Technology Dept; [3]Faculty of Veterinary, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
*Email: tcloh@putra.upm.edu.my

Colnago and Jensen (1) reported that adding putrescine to protein deficient diets failed to affect the performance parameters in broiler production. In contrast there is some evidence that dietary induction of hepatic ornithine decarboxylase (ODC, the key enzyme in polyamines and putrescine biosynthesis) is depended on the nutritive value of dietary protein (2, 3).In other study, Suka et al (4) reported thatODC is induced after feeding with different dietary protein contentin male mouse kidney. Synthesis of polyamine is a prerequisite for the rhythmic growth of liver in rat, also there is a correlation between the hepatic ODC level and the liver relative growth rate (2). Increased influx of amino acids into liver is a critical factor for dietary induction of ODC. Both glucagon and insulin play important role in stimulatingamino acid influx (5).Thus, there are some relationships between polyamines and pancreatic hormones.In chicken, excess dietary lysine increased renal concentrations of ornithine due to induction of arginase and consequently the putrescine level increased (6).

In view of the contrary information about the relationship between dietary protein and polyamines, more investigation is required to study their actual relationships in broiler chicken. In this experiment, the effects of dietary crude protein (CP) and methionine (Met) levelwere investigated with or without putrescine (Put) supplementation on the performance and nutrients digestibility rate in broilers.

The treatments include factorial combination of 2 CP levels (low and normal), 2 Met levels (low andnormal and 2 PUT supplemental levels (0 & 0.03%) producing 8 different diets. Each treatment consisted of 6 replicates containing 6 chickens each. Body weight and feed intake were measured weekly.At 24 days of age, 3 chicks per treatments were caged individually and kept 4 days for fecal collection. TiO2 was added (0.25 %) to each diet group to chicken as indicator. The feces and diet samples were analyzed for dry matter, ash, crude protein, calcium, phosphorus and gross energy in order to calculate digestibility of nutrients.

Table1 shows the effects of treatments on performance parameters. Protein deficient diets caused decreased feed intake and growth rate significantly ($P<0.05$) at the age of 21-42. This result shows that protein deficiency is important for growing. Interaction effects of treatments showed that body weight gain of normal protein group decreased significantly ($P<0.05$) when methionine is insufficient. Supplementation ofputrescine to low protein group improved body weight gain significantly ($P<0.05$) but FCR was affected negatively. This information is in contrast withthe results obtained by Colnago and Jensen (1), who did not observe positive effects of putrescine supplementationbut in agreement with Noguchi et al (2) and Murakammi et al (3).
Protein deficient diet causedlower ashdigestibility butmethionie deficient diets increased the digestibilityof ash significantly ($P< 0.05$). Putrescine fortified diets decreased ash digestibility significantly ($P< 0.05$). Interactions between treatments showed that dry matter digestibility in normal methionie diets is decreased significantly

(P< 0.05) when protein is insufficient. Adding putrescine to low methionine diets increased ash digestibility significantly (P< 0.05).

Table 1: Simple effects of treatments (protein, methionine and putrescine) on performance parameters of broilers of different periods

	0-21				21-42				0-42			
	FI[1] g/h/d	dBWG[2] g/h/d	FCR[3]	BWG[4] g	FI g/h/d	dBWG g/h/d	FCR	BWG g	FI g/h/d	dBWG g/h/d	FCR	BWG g
Protein*												
L	52.5	30.8	1.7	657	136.5[b]	59.5[b]	2.29	1200[b]	90.1	89.92	2.06	1883
N	51.8	30.3	1.7	657	143.8[a]	65.5[a]	2.2	1319[a]	92.6	92.6	2.01	1971
SEM	0.69	0.47	0.01	9.4	2.62	0.98	0.04	23.4	1.5	1.49	0.02	23.7
Methionine*												
L	51.8	29.9	1.7	643	142.5	62.5	2.28	1268	92.2	92.0	2.07	1912
N	52.5	31.2	1.6	671	137.8	62.5	2.21	1251	90.5	90.5	2	1942
SEM	0.69	0.47	0.01	9.4	2.62	0.98	0.04	23.4	1.5	1.49	0.02	23.7
Putrescine %												
0	52.1	30.6	1.7	657	142.2	63.2	2.25	1262	92.1	92.1	2.04	1937
0.03	52.2	30.6	1.7	657	138.8	61.8	2.24	1257	90.6	90.5	2.03	1917
SEM	0.69	0.47	0.01	9.4	2.62	0.98	0.04	23.4	1.5	1.49	0.02	23.7

Means ± SEM in the same column with non-common superscript are significantly different. (P<0.05)
*Protein and Methionine are balance to contain low(L) and normal (N) levels as in NRC
[1,2,3,4] Fi=Feed intake; dBWG=daily body weight gain; FCR= feed conversion ratio; BWG= total body weight gain.

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EFFECTS OF LIGHT AND DARK PERIODS DURING THE HOT PERIOD OF THE DAY ON THE PERFORMANCE OF LAYING HENS

H. Kassim*
Department of Animal Science, University Putra Malaysia, Serdang, Selangor.
*Email: kassim@putra.upm.edu.my

External conditions such as weather and climate can affect animal production and physiological responses. However, with poultry the environment usually refers to external conditions which are found in the bird microenvironment. Factors such as nutrition, water, air, temperature, humidity, light, disease, social relationship, sound and waste need to be controlled so that birds can maintain normal physiological functions and produce meat or eggs at its maximum rate. The most important physiological response of poultry to the environment is the constant maintenance of a homeothermic state during exposure to extreme ambient temperatures.

During exposure to hot ambient temperatures, poultry have a difficult problem keeping themselves cool and maintaining homeothermic body temperature. Since birds do not sweat, they must rely on evaporative cooling (panting) to keep themselves cool. The increased rate of panting produces respiratory alkalosis of the blood (1). This physiological response is characterized by an increase in blood pH, along with a decrease in blood CO_2 concentration. This upsets the blood acid-base balance and produces a decrease in blood calcium and bicarbonate which are necessary for the production of strong egg shells. The recommended temperature for laying hens is usually around 21-24^oC (2). The objective of this study was to evaluate the effect of darkness in the management of the adverse effect of high ambient temperature on the performance of laying hens.

Forty mature 28-week old Hisex Brown layers were divided into two equal groups of 20 and were caged individually. The two groups of birds were housed in two separate rooms; dark-treated and light-treated (control). The photoschedule was synchronized to ambient temperature so that the dark period occurred during the period of high ambient temperature (35^oC) which was between 1200 and 1600 hours. The dark period of the dark-treated group started from 1200 to 2000 hours, and the light period from 2000 to 1200 hours. For the control group, the dark period was from 2400 to 0800 hours and the light period from 0800 to 2400 hours, as shown in Figure 1.The light and dark periods were controlled automatically. The birds were continuously exposed to the treatment for a period of 18 weeks. Birds were fed a commercial layer diet and water was provided *ad libitum*. The parameters recorded were rectal temperature, feed intake, egg production and egg shell thickness which were measured weekly. Blood samples were collected for blood gas analysis (pCO_2, pO_2) and blood chemistry (pH, pCO_2, HCO_3, Ca, P and cholesterol).

Synchronization of the dark period of the photoschedule with the hot period of the day significantly reduced hyperthermia and increased feed intake, egg production and egg shell thickness (Table 1). However, egg weight and feed conversion were decreased. The heart weight and plasma cholesterol concentration of laying hens exposed to high ambient temperature during the light period were significantly increased (Tables 2, 3, 4).

Management technique to increase nutrient intake during heat stress would minimize the drop in production efficiency. The easy way to increase nutrient consumption is to feed the birds at the time of day when feed consumption is highest

(the light-to-dark cycle results in a u-U-shaped feed consumption curve). If birds are fed during the cool part of the day, feed consumption would be higher. Birds should not be fed during the afternoon in periods of hot weather since this will increase the amount of body heat that they must dissipate and thus increases the potential for heat prostration.

Fig 1: Diagrammatic representation of the photoschedule of Dark Treated and the Light Treated Hens (control) throughout the 24 hours Light-Dark cycle

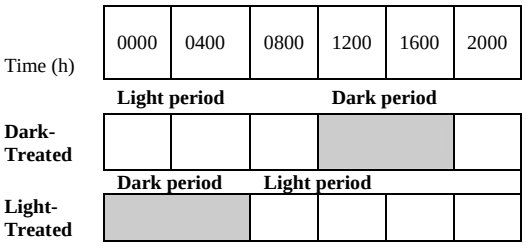


Table 1. The Effects of dark and light treatments on performance of laying hens exposed to high ambient temperatures (35°C)

Parameter	Light-Dark Cycle		p-value
	Dark	Light (control)	
Feed intake, g/h/d	91.6 ± 5.70	88.0 ± 6.15	0.0001
Feed conversion (ratio)	1.9 ± 0.14	1.86 ± 0.16	0.03
Egg production %	85.2 ± 4.1	82.1 ± 4.2	0.03
Egg weight (g)	56.9 ± 0.44	57.6 ± 0.63	0.003
Egg shell thickness (mm)	0.42 ± 0.02	0.39 ± 0.03	0.001

Table 2. The effect of dark and light treatments on body temperature and blood gas parameters in laying hens at high ambient temperature

Parameter	Light-Dark Cycle		p-value
	Dark	Light	
Rectal temperature (°C)	41.2 ± 0.23	41.4 ± 0.21	0.014
Blood pH	7.4 ± 0.02	7.4 ± 0.03	0.64
pCO_2	36.1 ± 4.90	32.4 ± 4.90	0.02
HCO_3	23.8 ± 2.86	21.0 ± 2.27	0.01
pO_2	46.0 ± 5.83	43.1 ± 6.48	0.20
O_2 saturation	82.7 ± 5.72	79.1 ± 7.99	0.09

Table 3. The effect of dark and light treatments on blood biochemistry (cholesterol, calcium and phosphorous) in laying hens at high ambient temperature

Parameter	Light-Dark Cycle		p-value
	Dark	Light	
Cholesterol (mmol/l)	2.3 ± 0.91	3.9 ± 0.91	0.02
Calcium (mmol/l)	5.7 ± 1.75	6.0 ± 1.68	0.5
Phosphorous (mmol/l)	0.9 ± 0.46	1.1 ± 0.50	0.1

Table 4. The effect of dark and light treatments on visceral eight of laying hens at high ambient temperature

Parameter	Light-Dark Cycle		p-value
	Dark	Light	
Live weight (g)	1639 ± 151.1	1673 ± 136	0.5
Liver weight (g)*	28.5 ± 3.56 (1.74)	26.0 ± 3.00 (1.56)	0.07
Oviduct weight (g)*	133.6 ± 12.01 (8.15)	120.0 ± 31.34 (7.17)	0.12
Spleen weight (g)*	2.8 ± 0.67 (0.17)	2.4 ± 0.69 (0.14)	0.14
Heart weight (g)*	6.3 ± 1.17 (0.38)	7.2 ± 1.24 (0.43)	0.05

* Relative weight is the weight of the organs as percentage of live weight

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CORRELATION OF UTERUS COLOR AND TURGIDITY ON CONCEPTION RATE IN DORPER SHEEP

Y. Jasmi[1]*, A.R. Mohd Fadzil [1] and Johari Jiken[2]
[1]Strategic Livestock Research Centre
Stesen MARDI Kluang, P.O. Box 525, 86009 Kluang, Johor, Malaysia.
[2]Strategic Livestock Research Centre, Malaysian Agriculture Research and Development Institute (MARDI), P.O. Box 12301, 50774 Kuala Lumpur, Malaysia.
*Email: jasmi@mardi.gov.my

Dorper sheep has been identified as a potential breed for meat production in Malaysia. Some of the characteristics of Dorper sheep are good mutton production, adaptability, hardiness, weld utilization, short mixed of wool and hair and good mothering ability (1). There is high demand for breeder animals and one of the ways to improve and spread the genetic of the breed is by artificial insemination (AI). The aim of the study was to determine the correct time to inseminate Dorper ewes after standing heat by observing the turgidity and color of the uterus by using laparoscopic technique. This paper reported the condition of the uterus, 12 and 24 hours after standing heat.

A total of 54 Dorper ewes aged between 24 and 30 months were randomly selected. The ewes were reared under semi-intensive management where they were kept in the shed at night and allowed to graze from 10.00 am to noon during the day. Concentrate feed was given to the ewes in the shade at the rate of 200-300 grams per animals per day. The ewes also received routine de-ticking, drenching and vaccination for herd health maintenance. Estrus synchronization was performed on the ewes by placing CIDR with 0.3 gram progesterone for twelve days. On removal, 125 microgram of prostaglandin was injected intramuscularly. Standing heat was observed and the time recorded. The ewes were inseminated with 0.25 ml frozen semen using laparoscopy technique either 12 hours or 24 hours after standing heat when turgidity and color of the uterus was pink to red. Pregnancy diagnosis using ultrasound method was carried out after 2 months after insemination.

Data collected included the color and turgidity of the uterus at 12 and 24 hours after standing heat and the number of pregnancy after insemination were analyzed using GLM procedure, SAS Statistical software programme.

Table 1: Ratio and percentage of ewes that were detected pregnant two months following insemination.

Color of uterus	Ratio of ewes pregnant	% Pregnant
Pale	10/26	38
Pink	1/5	20
Reddish	21/23	91

The number of ewes that responded to the synchronization by showing standing heat was 52 (96%) animals. The mean time for detecting standing heat in ewes after CIDR removal was 46.53 ±1.84 hrs with a range of 33 to 63 hrs. All ewes showed turgid uterus at 12 and 24 hours after standing heat. The color of the uterus 12 hours after standing heat was 26 (49%) pale, 5 (9%) pink and 23 (43%) reddish, 24 hrs after standing heat 16 (30%) pale, 2 (4%) pink and 36 (68%) reddish in color.

The relationship between the colors of the uterus 12 hours after standing heat with the pregnancy rate is summarized in Table 1. Ewes with pale uterus at 12 hours and artificially inseminated at 24 hours resulted in 38% pregnancy. Ewes with pink uterus at 12 hours and artificially inseminated at 12 hours showed 20% pregnancy while ewes with reddish uterus at 12 hours when inseminated at 12 hours resulted in 91% pregnancy.

The result showed that higher percentage of pregnancy occurred when AI was performed on uterus that was reddish in color and turgid. The time of AI, 12 and 24 hours after standing heat observed in this study appear to be similar to the average time of 15-20 hours reported by other workers (2). Further work need to be done to determine the correlation of the color of the uterus and the conception in Dorper sheep.

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SERVING CAPACITY TEST OF SEXUALLY-INEXPERIENCED BOER BUCKS

N.S.Mariani[1*], H.Yaakub[2], Yusof Daud[3], J.A.Johari[4], S.Shanmugavelu[4]
[1]Strategic Livestock Research Centre, Station MARDI Kemaman, Terengganu.
[2]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor.
[3]Biotechnology Center, JPHPT Keningau Farm, Sabah.
[4] Strategic Livestock Research Centre, MARDI, 43400 Serdang, Selangor.
Email: *niksiti@mardi.gov.my

Bucks play a major role in any goat breeding program. One of the methods employed to select potential bucks for breeding purpose is the serving capacity test. Serving capacity test is an evaluation of the sexual performance (ejaculations/male/mount time) of a male animal. High performing buck will be able to ejaculate and shift females at a faster rate and therefore inseminate many females per unit time. There was a positive association between buck with high scores for sexual performance and doe fertility (2, 3, 4) which eventually influenced flock fertility. Therefore, assessing sexual activity of bucks using the serving capacity test is a prerequisite for its selection for breeding in the goat industry.

Ten healthy Boer bucks (1½ years) with no previous experience of sexual activity were randomly selected and kept in individual pens. Feed was offered at 4% bodyweight (DM basis). Each buck was allowed to mate naturally with a restrained female (on oestrus) for 30 minutes. Data on serving capacity test were collected every 2 weeks for 5 consecutive weeks. All occurrences of mount attempts, mounts with and without ejaculation and the times at which each event occurred were recorded. Latency to first mount, latency to first ejaculation (bucks without ejaculating were assigned a score of 1800 - sec) (1) and refractory period was calculated. Buck efficiency was calculated by dividing the total number of ejaculations by the total number of mounts with and without ejaculation.

Table 1: Serving capacity test parameters of bucks for five weeks (mean±sem).

Weeks	Latency to first mount (sec)	No. of mounts & mounts attempts (n/30 min)	Latency to first ejaculation (sec)	Refractory period (n/30 min)	No. of ejaculation (n/30 min)	Mating efficiency
1	106.6±34.8^{a}	28.4±4.3^{ab}	1617.6±159.6^{a}	1622.4±157.2^{a}	0.3±0.2^{b}	0.08±0.07^{bc}
2	31.8±7.6^{b}	34.3±4.3^{a}	783.7±248.3^{b}	641.6±259.7^{b}	1.2±0.4^{b}	0.04±0.01^{c}
3	26.3±3.5^{b}	33.0±6.9^{a}	332.5±171.9^{c}	468.0±183.5^{bc}	1.5±0.4^{b}	0.06±0.02^{bc}
4	34.8±4.1^{b}	24.5±4.3^{ab}	161.5±75.8^{c}	89.2±34.2^{c}	3.0±0.4^{a}	0.19±0.05^{ab}
5	43.2±6.4^{b}	19.2±3.3^{b}	124.2±17.4^{c}	129.5±70.9^{c}	4.3±0.8^{a}	0.25±0.05^{a}

^{abc} Means value in the same column with different superscripts is significantly different ($P<0.05$); Sem standard error of mean.

Results showed that there were significant differences ($p<0.05$) of week for the means latency to first mount, number of mount and mounts attempts, latency to first ejaculation, refractory period, number of ejaculation and mating efficiency. Bucks showed an equivalent improvement in sexual performance from week 1 to week 5. At the first exposure, bucks took longer time for latency to first mount, latency to first ejaculation, refractory period and lower number of mount and number of ejaculation

which in turn resulted in low level of mating efficiency (1). Only 2 of 10 bucks ejaculated at the first week. Factors such as shyness, being strange to the testing environment and the presence of the observer nearby might have contributed to the poor performance of the bucks at the first week. At the end of the study all 10 bucks ejaculated successfully. The number of ejaculation and mating efficiency increased over the values at the beginning of the study. It was noted that experience has a decisive effect on the ability of bucks to properly mount and successfully ejaculate. However it cannot be denied that the method of restraint of estrous females may also have some influence on the sexual behavior of bucks.

The results suggest that Boer bucks in this study have good libido and high performing males. However, it is essential that the bucks have had adequate serving capacity test before they attain full sexual performance (1). This is evident from the reduction in the latency to first ejaculation time (Table 1) with a low standard error of mean. Sexual experience may provide opportunity for bucks to learn and recognize important behavioral cues from the females. Further studies should be carried out to correlate serving capacity with, sperm quality and serum testosterone level in order to reflect the actual reproductive potential of Boer bucks.

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EFFECTS OF POST ACTIVATION TREATMENT WITH TRICHOSTATIN A ON EPIGENETIC STATUS OF CLONED BOVINE EMBRYO

Sh, Jafari[1*], H., Yaakub[1], H., Wahid[2], N. Noordin[3], M.H., Nasr-Esfahani[4] M., Anjomshoaa[5,6]

[1]Department of Animal Science; [2]Department of Veterinary Clinical Studies; 3Department of Human Development and Growth, Universiti Putra Malaysia, Malaysia; [4]Royan Institute for Animal Biotechnology, ACECR, Isfahan; [5]Department of Anatomical Science, University of Medical Science, Rafsanjan; [6]Department of Medical Basic Science, Islamic Azad University, Khorasgan (Esfahan),Iran.
*Email: sh_jeferson@yahoo.com

Embryos from the somatic cell nuclear transfer (SCNT) procedure have a much lower chance of resulting in successful offspring (1). Insufficient epigenetic reprogramming has been thought to account for this phenomenon (2). In this regard, several deficiencies in epigenetic reprogramming of somatic nuclei after SCNT have been underlined (3). The differentiation of a somatic genome into the pluripotent state is a key event during reprogramming (4). One of the well known epigenetic modifications is histone acetylation (5). Acetylation of histone is associated with active chromatin configuration (6) and prevents the subsequent methylation of this lysine residue. This status can be manipulated by treatment with a histone-deacetylase inhibitor, trichostatin A (TSA). Therefore, the objective of the study was to improve nuclear reprogramming and increase the cloned blastocyst rate if correctly implemented.

The aspirated cumulus-oocyte complexes (COCs) from antral follicles of cow ovaries were then matured and denuded. Later, oocyte enucleation was performed on a pre-warmed microscopic stage as described (7). Prior to nuclear transfer, a single cell suspension of the adult fibroblasts was prepared. The SCNT embryos were cultured for seven days after activation, the immunostaining was conducted to detect 5-methyl cytosine and acetylated histone H3 on lysine 9 (H3K9) in developed blastocysts. Data were analyzed using one-way ANOVA (SPSS 16). The differences between experimental groups were considered significant at $P<0.05$.

The mean values of DNA methylation of the embryos were significantly greater ($P<0.05$) in the control compared to the TSA group, but the TSA group was significantly higher ($P<0.05$) than in the IVF embryos. The mean intensities of H3-acetylation was significantly lower ($P<0.05$) in the control compared to the TSA group, however the TSA group was significantly lower ($P<0.05$) than the IVF embryos.

The results in agreement with other studies indicated that the cloned blastocysts are generally hypermethylated of DNA and hypoacetylated of histones in comparison to blastocysts derived from IVF (8). Furthermore, it was indicated that treatment with TSA has the potential to modify the epigenetic status of the reconstructed embryos, though the acetylation status of these embryos were yet significantly lower than fertilized embryos. Importantly, direct TSA-mediated increase in acetylation levels of the embryo was concomitant with indirect decrease in methylation. It has been demonstrated that DNA-methylation and histone-acetylation are inter-associated pathways, resulted an important implications for study of normal development, somatic cell reprogramming and tumorigenesis (9).

Although it shows low efficiency of the somatic cell nuclear transfer (SCNT) procedure, the hypothesis that epigenetic marks of somatic donor cells are responsible for this inefficiency is proposed. Epigenetic marks such as DNA methylation and

histone acetylation are two major participants in nuclear reprogramming which can be used as indicators to assess SCNT efficiency.

In conclusion, the SCNT embryos shows both reduction and increase of DNA methylation and histone acetylation levels in the blastocyst stage of that treated with 0.05 µM Trichostatin A, a histone deacetylase inhibitor, after activation for 12 h, which is similar to IVF-derived blastocysts.

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REPRODUCTIVE PERFORMANCE OF KATJANG CROSSBRED FED A MIXTURE OF *GLIRICIDIA SEPIUM* AND GRASS (*ISCHAEMUM RUGOSUM*) UNDER INTENSIVE PRODUCTION SYSTEM

S. Sadi[1*], S. Shanmugavelu[2], A.R.Azizan[3], A. Dzulfazly[4], F.M. Abdullah[5], M. Wan Zahari[6] and K. Humrawali[7]

[1,2,3,4] Strategic Livestock Research Centre, [5]Bukit Ridan MARDI Research Station, [6]Formerly from Strategic Livestock Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), G.P.O.Box 12301, 50774 Kuala Lumpur, Malaysia, [7]Department of Agriculture Sarawak, 12-17th Floor, Menara Pelita, Tun Abdul Rahman Yakub Road, Petra Jaya, 93050 Kuching Sarawak.

*E-mail: sadi@mardi.gov.my

About 100 matured female (doe) goats (Katjang crossbred) and 4 matured bucks (Boer bred) were randomly assigned into 4 treatments. The bucks were rotated weekly among the treatment group. The treatment diet is presented in Table 1. The CP values of the mixed diet was 11-17% within the range of the 11-12% recommended by the National Research Council (NRC) (1) to meet requirements for moderate BW gains in goats. The gross energy (GE) of *Gliricidia sepium* mixed diet (D2, D3 and D4) was 17.46, 17.81 and 17.89 MJ/kg respectively, slightly higher than reported values of 17.3 MJ/kg. All goats were provided a concentrate supplement at the rate of 1.0% of their live weight in dry matter (DM). The concentrate supplementation was based on rice bran, palm kernel cake (PKC), urea, molasses, salt, limestone, vitamins and minerals. The feeding period was for 9 months to allow for one breeding cycle, kidding and weaning.

Table 1: Chemical composition of diets (DM basis)

Diet	D1	D2	D3	D4
G. sepium leaves (%)	0	30	50	70
Grass (%)	100	70	50	30
Parameters				
DM (%)	27.07	24.15	27.69	25.95
CF (%)	26.28	25.52	20.90	19.47
ADF (%)	37.04	35.11	32.79	34.01
NDF (%)	64.03	60.63	54.93	51.65
Ash (%)	7.73	8.10	8.60	7.94
EE (%)	1.30	1.56	1.56	1.78
CP (%)	10.98[a]	13.43[b]	15.59[c]	17.26[d]
Ca (%)	1.80	1.85	2.19	2.16
P (%)	0.13	0.17	0.24	0.20
GE (MJ/kg)	17.22	17.46	17.81	17.89

D-Diet; DM-Dry Matter; CF-Crude Fibre; ADF-Acid Detergent Fibre; NDF-Neutral Detergent Fibre; Ca-Calcium; Cu-Copper; CP-Crude Protein; EE-Ether Extract; GE-Gross Energy; P-Phosphorus; [a-d] Rows with different superscripts differ significantly ($P<0.05$).

The reproductive performance of goat fed a mixture of *G. sepium* and grass (*Iscahemum rugosum* Salisb) under intensive production system is shown in Table 2. The number of single birth was higher ($P < 0.05$) in group D4. The neutral detergent fibre (NDF) concentration was within the range reported by Meissner et al (2). The

NDF (2) and acid detergent fibre (ADF) (3) may influence intake that affects BW gain. However, in an earlier study, the inclusion of *G. sepium* in the diet did not significantly influence body weight (BW) gain of goats (4). Furthermore, Hossain et al (5) noted that, BW of does is also an important factor affect birth rate. The absence of twins or triplets birth in the goats fed diet D4 cannot be ascertained from this study. It is possible that other factors such as size, health status of does (5) or some phytochemical components of *G. sepium* may have contributed which requires further inevstigations.

Table 2: Reproductive performance of goats fed a mixture of *Gliricidia sepium* and grass under intensive production system.

Diet	D1	D2	D3	D4
Bucks (Boer) (n)	1	1	1	1
Does (Katjang) (n)	25	25	25	25
No. birth				
Single	11^{a}	14^{b}	12^{a}	19^{c}
Twin	6^{a}	2^{b}	2^{b}	0^{c}
Triplet	1^{a}	1^{a}	0^{a}	0^{a}
Total of birth	18	17	14	19
Total of kid	26	21	16	19

^{abc}Rows with different superscripts differ significantly ($P<0.05$).

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PRODUCTION OF CLONED-GOAT EMBRYOS THROUGH INTERSPECIES SOMATIC CELL NUCLEAR TRANSFER (ISCNT) USING TWO DIFFERENT NUCLEAR TRANSFER METHODS

H.H. Soh, W.E. Wan Khadijah and *R.B. Abdullah
Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
*Email: ramli@um.edu.my

Production of cloned-goat embryos using the intraspecies somatic cell nuclear transfer is limited by low source of goat oocytes as the recipient cytoplast in Malaysia. Therefore, by using the cattle oocytes as recipient cytoplast in interspecies somatic cell nuclear transfer is the only alternative approach to produce large number of cloned-goat embryos and subsequently offspring at a rapid rate. The objective of this research was to compare the effect of nuclear transfer methods on iSCNT cloned embryos developmental competency involving: a) intracytoplasmic injection, and b) sub-zonal injection with electrofusion. In the former method, a single donor cell was directly injected into the oocyte cytoplasm. In the latter method, a single donor cell was placed at the perivitelline space of an oocyte and the injected oocytes were undergoing electrofusion to facilitate the donor cell fused into the oocyte cytoplasm.

Dominko *et al.* (1999) (1) reported the first iSCNT experiments on sheep-cattle, pig-cattle, monkey-cattle and rat-cattle using cattle as the recipient cytoplast with the blastocyst percentages of 13.9, 14.3, 16.6 and 0%, respectively. Cattle oocyte has been proven to serve as a universal recipient cytoplasm to produce iSCNT cloned embryos and offspring. From the studies, researchers have demonstrated that the cattle cytoplast is capable of reprogramming somatic cell nuclei from other species which including the horse (2, 3, 4), pig (5), tiger (6), buffalo (7, 8), gaur (9) and goat (10).

Briefly, the bovine ovaries were collected from local abattoir and transported to the laboratory within 2 to 3 hours in 0.9% NaCl. Bovine oocytes (n=586) were recovered by slicing technique where checkerboard incision was made to the entire surface of the ovary by using the blade inside the culture dish. After slicing, the cumulus-oocyte complexes (COC) were recovered and selected under a stereo-microscope. Oocytes with several compact layers of cumulus cells were selected and cultured in *in vitro* maturation (IVM) medium for 22 hours. After maturation, COC were denuded in 0.1% hyaluronidase to remove the cumulus cells. The matured oocytes (n=358) with polar bodies were selected for enucleation to remove the spindle. Goat foetal fibroblast cells (donor cells) were harvested and transferred into enucleated oocytes by using either intracytoplasmic injection (n=157) or sub-zonal injection with electrofusion (n=201). The reconstructed oocytes were activated and cultured in KSOM medium for *in vitro* embryos development at 38.5^{o}C and 5% CO_2 in humidified atmosphere for 8 to 9 days.

From the results obtained, the percentages for cleaved embryo and blastocyst formation for sub-zonal injection with electrofusion were higher than the oocytes which underwent intracytoplasmic injection (63.01% vs. 56.30% and 12.33% vs. 5.88%, respectively). In summary, the nuclear transfer using both methods of sub-zonal injection and intracytoplasmic injection shows satisfactory results with the former method was apparently higher in *in vitro* developmental competence in both cases. Therefore, to our knowledge, we believe this is the first report of producing cloned-goat embryos using goat foetal fibroblast cells as donor karyoplasts and cattle as recipient cytoplasts in iSCNT in Malaysia. With this encouraging finding, using goat-cattle in

iSCNT to produce goat embryos and offspring may offer a new approach to increase genetically superior goat population in Malaysia at a rapid rate to meet the goat meat and milk demand for the industry.

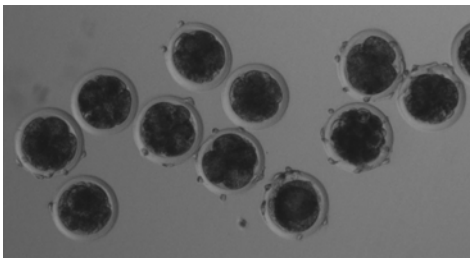


Figure 1: ISCNT cloned-goat embryos at 4- to 8-cell stages on day 3

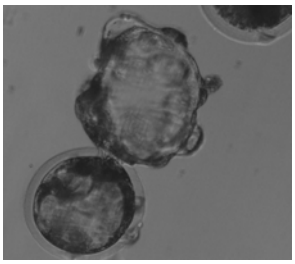


Figure 2: ISCNT cloned-goat embryos at blastocysts stage on days 7 to 8.

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PROTEIN FAMILY IDENTIFICATION OF SHEEP UTERUS EXPRESSED SEQUENCE TAGS (ESTs).

A.H.M.S. Suhaimi,[1*], I.A. Azlina Azma[2], A.R. Roziatul Erin[1], W. M. Z. Somarny[1], Mat Isa. Md. Noor[3], M. Abdullah Hashim[2], Sagiman, Md. Tasol[1], Z.A. Rabiatul Adawiah [4] and K. Musaddin[1]

[1]Strategic Livestock Research Centre, MARDI Headquarters, 43400 Serdang, Selangor.
[2] MARDI Muadzam Shah , Bandar Muadzam Shah,Pahang; [3]Malaysian Genome Institute, MTDC-UKM, Bangi, Selangor; [4]Biotechnology Research Centre, MARDI Headquarters, 43400 Serdang, Selangor.,
*Email : shusna@ mardi.gov.my

Expressed sequence tags (ESTs) are short DNA sequences of 200-800 nucleotide bases in length corresponding to gene transcripts of specific tissues. ESTs have many applications including gene discovery, complementing genome annotation, aiding gene structure identification and guiding single nucleotide polymorphisms (SNP) characterization (1). Currently, there are at least 69,535,030 ESTs submitted to the ESTs database at NCBI website. There are several approaches to analyze ESTs sequences. One approach is to predict the function of protein represented by these gene transcripts based on sequence similarity by alignment to Pfam, a comprehensive collection of protein domains and families (http://www.sanger.ac.uk/resources/databases/pfam.html). The current release of Pfam (22.0) contains 9318 protein families (2). This approach is suitable for species whose genome has not been fully sequenced such as sheep where genomic information is limited.

ESTs were generated from a cDNA library constructed from the uterine mRNA of a Malin sheep with high twinning rate. From the library, DNA plasmids were randomly isolated and sequenced as ESTs by automated sequencing. StackPACK 2.2 software was utilized to analyze the ESTs. Sequences were clustered using d2_cluster and assembled into consensus sequences using the application Phrap. CRAW was then performed to analyse sequence alignment. BLAST was used for database similarity search against the nucleotide sequence databases. Sequences were then translated and aligned to Pfam database for domain motif search at CLC Genomics Workbench (http://www.clcbio.com/) using InterProScan software (http://www.ebi.ac.uk/ Tools/pfa/iprscan/).

In total, 746 sequences were successfully sequenced. Upon vector cleaning, 342 single copy sequences known as singletons were identified. Sequences aligned to various species. The highest alignment is to *Bos Taurus* where 66.7% or 228 ESTs were identified as aligned. The next most frequent alignment was to *Homo sapiens* with 5.3% followed by horse, goat, pig and sheep. Other organisms constitute of 20.7% of the total number of ESTs aligned (Table 1). When all sequences were aligned to Pfam, 161 sequences aligned to known protein families or domains. In total, 16 protein families were identified with maximum of 12 hits and minimum of 1 hit (Figure 1). The three highest Pfam protein family identified were NADH-Ubiquinone oxidoreductase (complex I), chain 5 C-terminus (n=12), ATP synthase A chain (n=11) and Cytochrome C oxidase subunit II, transmembrane domain (n=9).

Highest alignment of sheep ESTs to *Bos Taurus* indicated high sequence homology between the two species suggesting many cow and sheep genes have high sequence similarity. Indeed, cross-species analysis has been applied to identify functions of many new genes (3). Alignment to the Pfam databases is a good approach as it can predict the function of gene transcripts represented by ESTs in species whose

genomic information is still scarce. This approach can be utilized in other high-scale projects in various animal species to identify gene functions that may be involved in causing diseases or other physiological disorders.

Table 1. Alignment of sheep uterine Expressed Sequence tags (ESTs) to different species.

Scientific Name	Common Name	No of ESTs	% of ESTs
Bos taurus	Cow	228	66.7
Homo sapiens	Human	18	5.3
Equus caballus	Horse	10	2.9
Capra hircus	Goat	7	2.0
Sus scrofa	Pig	5	1.5
Ovis aries	Sheep	3	0.9
Other organisms		71	20.7
Total		342	100.0

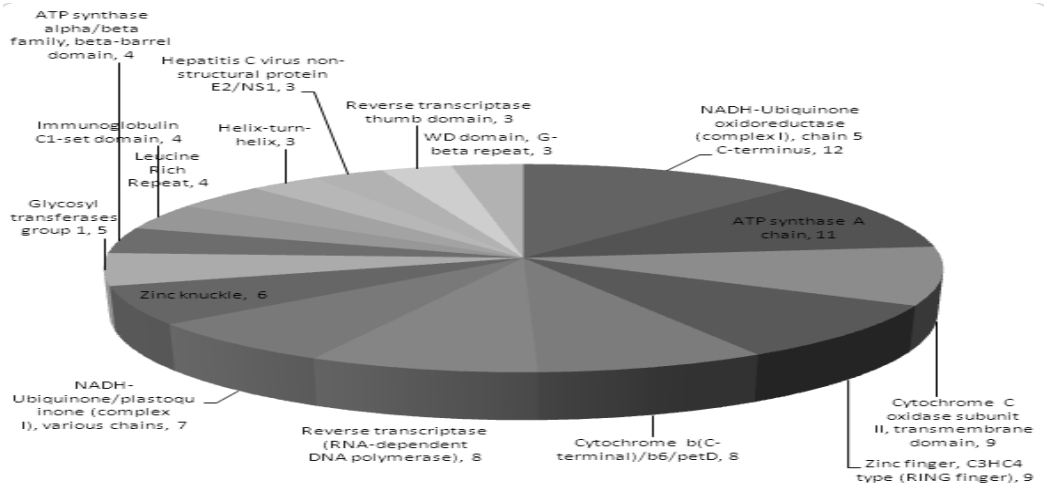


Figure 1. Protein domains and families identified in Malin sheep ESTs

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EFFECT OF VITAMIN E AND SELENIUM SUPPLEMENTATION ON OOCYTE QUALITY AND QUANTITY OF BOER CROSSES

A. Anna Aryani[1*], H. Yaakub[2], B. Abdul Rashid[3], and A.R. Alimon[2]
[1]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Kuala Lumpur
[2]Department of Animal Sciences, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Kuala Lumpur; [3]Livestock Consultant, Sabah Land Development Board (SLDB), Sabah
*Email: annaaryani@putra.upm.edu.my

Reproductive performance of livestock is determined by four factors which are genetic merit, environment, nutrition and management. However, nutrition factors are perhaps the most crucial in their direct affects on the reproductive phenomenon. The needs of Selenium (Se) are found to be related to the Vitamin E level in the diet. Vitamin E shares its antioxidant function with Se, and these nutrients appear to have mutual sparing effect on one another. Studies have shown that micronutrients and Vitamins effects and acts directly on the gonads and other reproductive organs. Studies on using Se and Vitamin E have been conducted in evaluate the meat quality of sheep (1) and lambs (2); milk production in heifers during late gestation stage (3); and these trace mineral and Vitamin also are able to acts directly on the reproductive organs (4, 5). An increased in the incidence of oestrus, fertility and prolificacy were reported in ewes received that Vitamin E together with Se (5). Only a few studies on the effect of Se and Vitamin E are found in the reproduction area in goats and the studies are either on Vitamin E or Se. The objectives of this study are to evaluate the quality and quantity of oocytes in Boer crosses goat supplemented Vitamin E and Se in the diets.

Twenty-eight matured female Boer Crosses with bodyweight ranges between 20 to 25 kg were used. All does were kept in a group at Field Two farm, Universiti Putra Malaysia. The does were fed on basal diet (concentrate and forage; free access to salt lick and water) during 14 days of adaptation period. The does were randomly divided into four treatment groups and received either i) 0.0IU Vitamin E and 0.0mg/kg DM Se (VitE0-Se0 or Control; n=10); ii) 3.0IU Vitamin E and 0.0mg/kg DM Se (VitE3-Se0; n=10); iii) 0.0IU Vitamin E and 2.0mg/kg DM Se (VitE0-Se2; n=9); or iv) 3.0IU Vitamin E and 2.0mg/kg DM Se (VitE3-Se2; n=10). The does were fed on the dietary treatments for 3 months slaughtered at the end of the experiment. Both ovaries were collected within 30 minutes post slaughter and transported to the laboratory at 35-37 ˚C within 2-3 hours. Slicing was conducted to obtain the Cumulus Oocyte-Complexes (COC). Recovered COCs were graded depends on the status of the cumulus cells (Grade A oocyte - more than 5 compact cumulus cells; Grade B - 3 to 5 layers of cumulus cells; Grade C - 1 to 2 layers of cumulus cells and Grade D - naked oocytes or COC with incomplete cumulus). The treatment effects on number of oocytes and quality of oocyte were analysed using One-Way ANOVA. The SPSS program (Version 18.0; SPSS, Chicago, IL) was used for all analyses.

In Table 1, there was significant higher ($P<0.05$) in the number of Grade A oocytes in VitE3-Se2 group compared with the other groups. The percentages of Grade A oocytes for VitE0-Se0, VitE3-Se0, VitE0-Se2 and VitE3-Se2 was 23.9±5.97, 44.0±3.45, 40.0±2.50 and 49.0±4.17 percent, respectively. Meanwhile, there was a significantly lower ($P<0.05$) in the number of Grade C oocytes in VitE0-Se2 group compared to VitE0-Se0, VitE3-Se0 and VitE3-Se2 groups, where the percentage of Grade C oocytes for VitE0-Se0, VitE3-Se0, VitE0-Se2 and VitE3-Se2 was 27.6±7.06, 22.7±3.86, 7.9±2.74 and 27.6±2.53 percent, respectively. There was no significant different ($P>0.05$) between all treatment groups on the number of Grade B and Grade D

oocytes. Meanwhile, the total number of oocytes in VitE3-Se2 was significantly higher (P>0.05) when compared to VitE0-Se0, VitE3-Se0 or VitE0-Se2.

Table 1: The effect of dietary supplement of Selenium and Vitamin E on the quality of oocytes in goats.

Treatment groups	Mean (±se) number of oocytes				
	Grade A	Grade B	Grade C	Grade D	Total
VitE0-Se0	4.7±1.38[a]	2.5±0.81[a]	4.8±1.58[b]	6.7±3.43[a]	18.7±3.43[a]
VitE3-Se0	9.7±0.84[b]	3.2±0.70[a]	4.8±0.65[b]	4.7±1.38[a]	22.3±2.22[a]
VitE0-Se2	6.7±0.80[ab]	4.2±1.11[a]	1.3±0.49[a]	4.7±0.67[a]	16.8±2.07[a]
VitE3-Se2	16.3±1.99[c]	4.5±0.76[a]	3.5±1.06[ab]	9.2±1.68[a]	33.5±3.03[b]

[a,b,c] Values within columns with different superscripts are significantly different (P<0.05)

In conclusions, the supplementation of Vitamin E and Se showed no effect on the number of Grade B and D oocytes but supplementation of 3.0IU Vitamin E and 2.0mg/kg DM Se has improved the quality and the total number of oocytes.

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EFFECT OF FAT SUPPLEMENT ON REPRODUCTIVE PERFORMANCE OF BRANGUS CROSS FEMALE CATTLE

I. Ismail[1*], M. Khairul Izwan [2], M. Hilmi[1] and A. R. Alimon[1]
[1] Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, [2]Institute of Tropical Agriculture, Universiti Putra Malaysia
*Email: isid@agri.upm.edu.my

Fat supplementation has become one of the methods to increase animal production in ruminants. Increasing dietary energy density by feeding supplemented fat may improve reproductive performance in cattle (1). Supplemented fat will be regulated in the body and stored as adipose tissues. These adipose tissues were believed to become the primary source of leptin hormone secretion (2) which is involved in many physiological control systems including those that determine growth, development, body composition, milk production and reproduction (3). Animals receiving supplementation of dietary fat tend to increase the size of preovulatory follicles (4). Apart from that, leptin has been proposed as one of the hormones that can influence reproduction (5) and appears to affect the central reproductive axis through its own receptors and the neurotransmitter, neuropeptide Y (NPY) (6). The objectives of this study are to determine the effect of dietary fat supplement on reproductive performance of Brangus cross female cattle and to investigate the link between leptin concentration and fat supplement to reproductive and general performance of Brangus cross female cattle.

Heifers and primiparous Brangus cross cattle (n=18) were randomly assigned to receive 0% (control), 2% or 5% dietary fat (Megalac®). The size of ovulatory follicles were recorded twice a day through transrectal scanning using Aloka® SSD500 scanner with 7.5 MHz linear array transducer during synchronization, at first and second normal estrous of each group until ovulation. The size of ovulatory follicles was measured using electronic caliper and frozen images were drawn on the follicle map. Weight of newborn calf was taken within 24 hours after birth. The groups were weighed and bled at weekly intervals from day 7 to day 440. The blood was collected into plain Vacutainer® tube from the jugular vein. Samples were stored overnight in 4^{o}C fridge before centrifuged at 700 x g for 20 minutes. Progesterone concentration was determined using Progesterone RIA kit (Diagnostic System Laboratories, Inc. Texas, USA). Leptin concentration was measured using the Multi-Species Leptin RIA kit (Millipore Corp. Billerica, MA, USA) and determined using gamma counters. Size and growth rate of follicles, highest peak of progesterone and leptin concentration were analyzed using GLM procedures of SAS (SAS inst. Inc., Cary, NC).

Size of ovulatory follicles between three periods of transrectal ultrasonography was found to be similar within groups ($p>0.05$) but the 5% group tend to have greater mean size of ovulatory follicles compared to other groups ($p<0.05$). Growth rate of follicles and the highest peak of progesterone concentration were not different between groups ($p>0.05$). The calving rate and conception rate was 33.33%, 16.67% and 50% for control, 2% and 5% group, respectively. The 5% group tend to have greater mean (27.67 kg ± 12.39) for total weight gain three months after calving. The 2% group gains only 2.0 kg and the control group lost their body weight (14.5kg±8.5). Mean daily weight gain for non pregnant cattle was 0.368 kg ± 0.1, 0.347 kg ± 0.03, and 0.42 kg ± 0.3 for control, 2% and 5% group, respectively. Mean daily weight gain for pregnant

cattle was 0.48kg ± 0.02, 0.38 kg ± 0.05 and 0.52 kg for control, 2% and 5% group, respectively. Mean daily weight gain of calves for control and 2 % groups were 0.66kg ± 0.04 and 0.62 kg, respectively. Mean days of first detected estrus were 84 d, 79 d and 90 d for control, 2% and 5% group, respectively. There was no significant difference detected in growth rate of the follicles and highest peak of progesterone concentration. Although the comparisons between groups were not significant, the 5% group tend to give better result compare to the control group. Generally, group supplied with higher amount of Megalac® have improved their reproductive performance and general performance in terms of ovulatory follicle size, growth rate of follicles, level of progesterone concentration, conception rate, calving rate, total weight gain during postpartum period and daily weight.

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SEMEN CHARACTERISTICS OF LOCALLY-BORN FULLBLOOD BOER BUCKS

K. Musaddin[1*], A.R. Azizan[1], J. Saadiah[1], H. Ajis[2] and H. Rozali[2]
[1] Strategic Livestock Research Centre, MARDI, P.O. Box 12301, 50774 Kuala Lumpur.; [2] MARDI Research Station Kluang, Johore
*Email: musaddin@mardi.gov.my

MARDI imported its first batch of purebred Boer goats from South Africa in 2001. This was followed by subsequent importation of fullblood Boers from Australia and later purebred Boers from Malawi. These imported goats formed the Boer nucleus herd at MARDI Research Station in Kluang, Johore. The locally-born male and female fullblood Boer goats were evaluated for growth and reproductive performance (1). As it was very expensive to purchase quality fullblood Boer breeding bucks from Australia or South Africa, a programme was initiated to evaluate and identify locally-born fullblood Boer bucks as potential studs. Thus, the objective of this study was to evaluate the semen characteristics of locally-born fullblood Boer bucks.

The bucks which were about 18-24 months old during the semen collection and evaluation period were reared in an intensive system where they were kept indoor most of the time. Oil palm frond based-total mixed ration (14.9% CP and 9.1MJ/kg ME) was fed daily to the bucks at a rate of 3% body weight. Water and mineral salt licks were provided ad libitum. Semen samples were collected from seven locally-born fullblood Boer bucks using warmed water filled artificial vagina. Estrous female was used as teaser for mounting. Semen collection was carried out in the morning, three days a week for two weeks and each ejaculate was kept in warm water at 38-39°C. The ejaculate was evaluated for colour (visual assessment as an indication of the semen density and possibility of semen contamination), volume (measured by calibrated semen collecting tube), motility wave score of 1-5 (microscopically evaluated), percentage of progressive sperm motility (microscopically evaluated), percentage of life sperm and percentage of normal sperm (microscopic evaluation of eosin-nigrosin stain-sperm), sperm concentration (hemocytometer) and libido (reaction time from introduction to ejaculation in seconds). ANOVA using the General Linear Models procedure (SAS 9.1; 2002-2003, USA) was carried out on the sperm parameters measured to determine the differences between individual Boer bucks at $p<0.05$.

The results of the present study are shown in Table 1. The ejaculate volume which ranged between 1.13±0.29 -1.62±0.22 was not significantly different ($P>0.05$) between the Boer bucks but was within the normal range reported for goats (2, 3, 4). Significant differences ($P<0.05$) in the motility wave and the percentage of progressive sperm motility were observed between the bucks. The upper range of the motility wave was higher than those reported in earlier study (5). The sperm concentration between the Boer bucks in the present study was significantly different ($P<0.05$) and was within the range reported for Boer bucks in South Africa (5, 6). Significant differences were observed in the percentage of live sperm of Boer bucks which ranged between 27.8±28.14 - 91.8±4.62. This range was wider than those reported in earlier study (5). The percentage of normal sperm was significantly different ($P<0.05$) between bucks and it ranged from 64.17 to 92.5%. The libido or the reaction time based on the time taken to achieve ejaculation after introduction to a teaser varied significantly between bucks. In conclusion, due to the variability in semen quality between locally-born fullblood Boer bucks in the present study, potential breeding bucks should be evaluated

thoroughly to determine their reproductive capability in order to improve or sustain goat farm productivity.

Table 1. Semen characteristics of locally-born fullblood Boer bucks.

Variables	Range ±SEM	Mean ± SEM	P<0.05
Volume (ml)	1.13±0.10 -1.62±0.20	1.44±0.08	NS
Motility wave (1-5)	0.17±0.17 – 4.33±0.21	2.26±0.31	*
Progressive sperm motility (%)	7.5±2.79 – 88.3±1.89	50.44±5.89	*
Live Sperm (%)	27.8±11.49 -91.8±1.89	68.6±5.49	*
Normal Sperm (%)	64.17±9.68 – 92.5±2.74	80.7±2.38	*
Sperm Concentration ($x10^9$/ml)	2.32±.0.44 – 5.85±0.5	4.28±0.28	*
Libido (sec)	28.2±6.71 – 74.2±8.16	45.4±3.87	*

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ARTIFICIAL INSEMINATION AT FIXED TIME IN BOER GOATS

B. Hasbudie[1], H.B. Abdul Mu'in[1], H. Yaakub[1]*, J. Theivanai[2], M.N. Ismawandi[3] and H.N. Quaza Nizamuddin[3],

Department of Animal Science, Faculty of Agriculture, University of Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; [2]Infoternak, Sungai Siput (U), and [3]Perak Department of Veterinary Services , Perak, Malaysia.

*Email: hali@agri.upm.edu.my

Artificial insemination (AI) is the process of transferring male gametes that have been collected artificially into the female reproductive tract. AI has important role for goat breeding under intensive system of production. Fixed timed AI (TAI) is commonly used in cattle (1), and it is important to increase the efficiency of goats breeding management. It can reduce labour cost for heat detection, kid management, AI and produce uniform kid. This study was to evaluate the time of AI in Boer goat after synchronisation.

Forty eight does were assigned to AI time after synchronisation. The does were AI either at 48 hours (AI48; n=24) or 54 hours (AI54; n=24) post CIDR withdrawal. All does were kept under intensive system throughout the experiment period and received feed and water *ad libitum*. Does were inseminated using intracervical insemination technique (2) with frozen-thawed semen. Pregnancy diagnosis was conducted post 30 days AI transrectal using ultrasonography with 7.5MHz transducer to detect the embryo. Blood samples was collected prior to AI and at day 30 post AI. Prior to centrifugation, blood samples were kept at room temperature for an hour and then in the refrigerator at 4ºC overnight and serum were transferred into the tube and stored at -20 ºC for further progesterone analysis. The serum progesterone concentration was determined using ELISA kit. Data on the number and the percentage of does pregnant and not pregnant were analysed using Chi-square analysis for significant differences and the progesterone concentrations were analysed using General Linear Model (SAS 9.0).

There was no significant difference (P>0.05) in the pregnancy rate of Boer does in the two treatment groups between AI48 and AI54 (Table 1).

Table 1. The number and the percentage of does pregnant and not pregnant on day 30 post AI insemination at 48 hours (AI48) and 54 hours (AI54) post CIDR withdrawal.

Treatment	n	No. of does pregnant (%)	No. of does not pregnant (%)
AI48	24	11 (45.8)	13 (54.2)
AI54	24	7 (29.2)	17 (70.8)

The mean progesterone concentration for the pregnant and not pregnant does before and after 30 days of AI for AI48 and AI54 was also no significant different (P>0.05) (Table 2).

The mean progesterone concentrations for pregnant does after 30 days were within the range 4.33 to 11.0 ng/mL (3). For non-pregnant does, the mean progesterone concentration was also no significant different (P>0.05) between before AI and after 30 days AI.

In conclusion, the pregnancy rate between fixed time AI at 48 hours and 54 hours after synchronised practice in this study was similar.

Table 2. The mean serum progesterone concentration (ng/mL ± SE) for does before and after insemination at 48 hours (AI48) and 54 hours (AI54) post CIDR withdrawal.

Treatment	Pregnant		Not pregnant	
	Before	After	Before	After
AI48	3.2 ± 0.85	4.7 ± 3.40	3.0 ± 0.50	4.5 ± 2.47
AI54	4.2 ± 1.04	4.4 ± 2.56	8.0 ± 4.89	6.0 ± 4.41

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EFFECTS OF SPERM MOVEMENT ON ICSI-DERIVED EMBRYO DEVELOPMENT IN GOAT

A.H. Nor Farizah, W.E. Wan Khadijah and *R.B. Abdullah
Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
*Email: ramli@um.edu.my

The role of sperm is important to determine the success of intracytoplasmic sperm injection (ICSI) procedure to fertilise the oocytes as well as to increase the fertilisation, cleavage, pregnancy and birth rates in goat. Sperm motility is correlated with ICSI outcomes as motility reflects sperm vitality to result in successful fertilisation and subsequent developmental competence (Goto, 1997). In a standard ICSI procedure, the sperm is normally immobilised prior to its injection into the oocyte. It has been shown that embryos can be obtained by injection of dead sperm in ICSI procedure, whereby the sperm nucleus is still intact and is able to fertilise the oocyte successfully. It has been shown that dead embryos correlated well with in order to reduce the possibility of degenerated DNA from totally dead sperm. However, in natural fertilisation in the female reproductive tract, sperm motility is important to ensure the success of fertilisation. Consequently, the sperm that are no longer motile will not be able to fertilise the oocytes. It is logical that low motility rate of sperm may contribute to lower rate of embryos obtained *in vivo* and *in vitro*. Therefore, in clinical and industrial situation, selection of highly motile sperm is preferred for ICSI procedures in mammalian species including goats. Due to lack of information as well as controversial issues regarding the sperm and ICSI performance relationship, therefore, it is timely to study factors such as sperm motility characteristics relating to ICSI performance in order to clarify the underlying factors so that this technique can be applied integratively with other technologies in advance farm management for the goat industry. Therefore, the objective of this study was to produce embryos via ICSI technique using slow and rapid movement of sperm in goats.

The source of oocytes was obtained from oocytes retrieval (OR) through the laparoscopic oocyte pick-up (LOPU) technique. The donor does were synchronised and superovulated using (CIDR) followed by injection of gonadotrophin (PMSG and Ovidrel) before oocytes retrieval by LOPU technique. A total 97 of matured oocytes were used for ICSI for this study and were randomly assign to combinations of sperm movement (slow and rapid) and grades of oocytes (A, B and C). The oocytes were matured and ICSI procedure was carried out according to the different combinations of treatments. Cleavage rates were obtained for each treatment combination by observation on the embryonic development daily using stereomicroscope for 7 consecutive days.

The results have shown that the percentage of cleaved oocytes in rapid sperm movement was higher than that of slow movement group (69.23% vs. 64.44%, respectively). The embryos developed *in vitro* until the morula stage (13.46% vs 2.22%, respectively). There was one embryo successfully developed until blastocysts stage from the rapid movement sperm (1.92%). In conclusion, both rapid and slow movement sperm could be used to produce goat embryos using ICSI procedure with rapid sperm movement gives better ICSI performance than slow movement.

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CHARACTERISTICS OF FRESH AND FROZEN SEMEN OF BOER BUCKS

S. Nikbin[1], J.M. Panandam[1*], H. Yaakub[1], M. Murugayah[2] and A.Q. Sazili[3]
[1]Department of Animal Science, Faculty of Agriculture; [2]Faculty of Veterinary Medicine; [3]Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[*]Email: jothi@agri.upm.edu.my

Goat buck fertility is highly influenced by semen quality which is described by a wide variety of sperm characteristics. Quality of the semen after freezing is important for success of artificial insemination (AI). Semen quality and sperm viability in the female reproductive tract are influenced by freezing and storage (1). The objective of this study was to evaluate the quality of fresh and thawed frozen semen of Boer goats.

Semen from 36 purebred Boer bucks of South African origin (2-3 years of age and of good health) were collected with the aid of a teaser buck into an artificial vagina (40-42°C) at 0900 to 1000 hours over a period of 8 months. Three ejaculates were obtained from each animal. Each collection was split into two parts. One part was diluted (1:100) with normal saline for fresh semen evaluation. The second part was diluted using commercial BioXcell (IMV, L'Aigle, France) to a final concentration of 200 $\times 10^6$ spermatozoa/ml, and filled into 0.25 ml straws with the aid of an automated straw filler. The semen-filled straws were equilibrated at 4°C for 3 hours and then exposed on nitrogen vapour for 4 min prior to being plunged into liquid nitrogen. One day after freezing, the semen was thawed at 36°C for 30 s. Both fresh and thawed frozen semen were analysed using computer-assisted semen analysis (HTM-IVOS, Hamilton-Throne Biosciences MA, USA). The semen volume (VOL), sperm concentration (SCON), general motility (MOT), progressive motility (PROG), percent displaying fast motility (FAST), medium motility (MEDM) and slow motility (SLOW), static sperm (STAT), average path velocity (VAP), straight-line velocity (VSL) and curvilinear velocity (VCL) were semen quality traits analyzed. The GLM procedure of SAS 9.1 was used for data analysis. Spearman correlation was used to test association between fresh and thawed frozen semen characteristics.

The statistical results revealed that season (SN) had no effect on motility traits (Table 1). This could be due to the similar daily photoperiod and temperature in Malaysia. Photoperiod and temperature are the principal factors affecting semen quality (2). Younger bucks had higher MOT during the rainy season. Season effect is a combination of many important factors which includes nutrition and disease (3). The younger bucks had higher values for the other motility and velocity traits as well ($P<0.05$). MOT, PROG, RAPD, MEDM and VCL of frozen semen were significantly ($P<0.05$) lower than those of fresh semen. Cryopreservation decreases sperm movement characteristics (4). The spearman rank analysis revealed a moderate and positive correlation between RAPD and PROG of fresh semen with MOT, PROG, RAPD, VAP, VSL and VCL of thawed frozen ($P<0.05$). No significant correlation was found between LIB, SCON and VOL of fresh semen with motility and velocity traits of frozen semen. The results were similar to Hashida *et al*. (1).

Semen freezing increases cellular damage, and consequently caused a reduction in semen quality. However, the fresh semen with higher progressive and rapid motility exhibit good semen quality even after freezing and may be suitable for use in AI.

Table 1. Effect of freezing (FR), age of buck (AGE) and season (SN) on semen quality traits.

Source	df	MOT (%)	PROG (%)	RAPD (%)	MEDM (%)	SLOW (%)	STAT (%)	VAP (µm/s)	VSL (µm/s)	VCL (µm/s)
FR	1	**	**	**	ns	**	**	ns	ns	*
AGE	1	**	**	**	*	ns	**	**	**	**
SN	1	ns	ns	ns	ns	ns	ns	*	*	ns
SN*FR	1	*	ns	ns	ns	ns	**	ns	ns	ns
AGE*FR	1	ns	ns	ns	ns	ns	ns	*	*	ns
SN*AGE	1	*	ns	ns	*	ns	*	**	*	ns

* - significant at P<0.05, ** - significant at P<0.01, ns – non significant; FR – freezing, SN - season

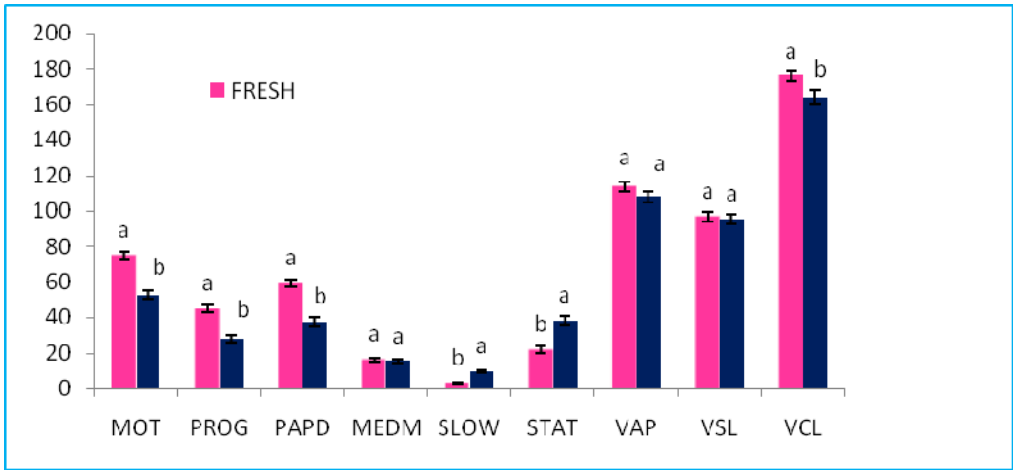


Fig. 1. Comparison of mean values for fresh and frozen semen quality traits Different superscript denote statistical significance (*P< 0.05*)

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HORMONES PROFILE OF SYNCHRONIZED COW

Syafnir[1], I. Noraida[1*], N.H. Hashida[2], T. Normala[3], Z. Hassan[3], M. Fuad[3] and A.R Hazudi [3]

[1]Institute of Biological Sciences, Faculty of Science,
[2]Centre For Foundation Studies in Science, University of Malaya, 50603 Kuala Lumpur, [3]Veterinary Services Department, Kelantan
*Email: aida@um.edu.my

Estrus synchronization involves controlling or manipulating the estrus cycle so that females in a herd express estrus at approximately the same time. Many modern techniques, such as controlled intravaginal drug release (CIDR) device, are used to synchronize the estrus and thereby improve reproduction in cattle. Synchronization of estrus in cattle would allow one to predict the time of estrus and organize a proper breeding program (1). The objective of this study was to characterized the hormone profiles during the estrus synchronization after CIDR insertion.

This experiment was conducted in private farms in Tanah Merah, Kelantan from June 2010 to August 2010. The study was conducted with daily temperature range from 24 to 32^{o}C. All cows were subjected to rectal examination to assess ovarian function. Only the non pregnant cattle with corpus luteum and body condition score between 3 to 7 according to the scale of 0 (most emaciated) to 9 (fattest) were chosen for this study. All cycling cattle received CIDR insertion (Day 0) containing 1.38 g of P4 (Eazi-Bred™ CIDR®; Pfizer Inc., New Zealand) which attached to estradiol benzoate capsule (Pfizer New Zealand Limited). The cattle were given 2 mg i.m injection of estradiol benzoate (EB) (Bomac Laboratories) after 24 h of CIDR removal (Day 8). Twenty four hour after EB injection (Day 9), a single insemination was carried out to each of the synchronized cattle using rectovaginal method. The frozen semen was provided by Kelantan Veterinary Services Department. Pregnancy diagnosis was carried out on day 60 post-insemination using rectal palpation.

Blood (10 ml) was collected at the jugular vein of the cow using jugular venipuncture (BD Franklin Lakes NJ USA). Blood samples were collected on day 0, 3, 7, 8, 9, 12 and 14 of the experiment to characterize progesterone and estradiol concentrations. All collected blood samples were immediately placed in the ice box and then centrifuged at 3,500 x g for 10 min. Serum was then decanted and stored at -20°C until assayed. Concentrations of progesterone and estradiol in serum were assayed using a RIA kits (Immunotech a Beckman Coulter Company).

The CIDR implants that were inserted on day 0 after blood collection had caused the progesterone level to increase immediately after implantation; the progesterone level decreased when the CIDR implants were removed (Figure 1). The mean progesterone level of the cows on day 0 was 3.16 ng/ml. The progesterone level reached the peak on day 3 (12.76 ng/ml). After CIDR removal on day 7, the level of progesterone started to decline and the lowest level was observed on day 14 (0.97 ng/ml). Estradiol level was 3.27 pg/ml on day 0 for all breeds. On day 8, that is after CIDR removal and estradiol benzoate injection, the level of estradiol started to increase and achieved the peak on day 9 (43.47 pg/ml) (Figure 2).

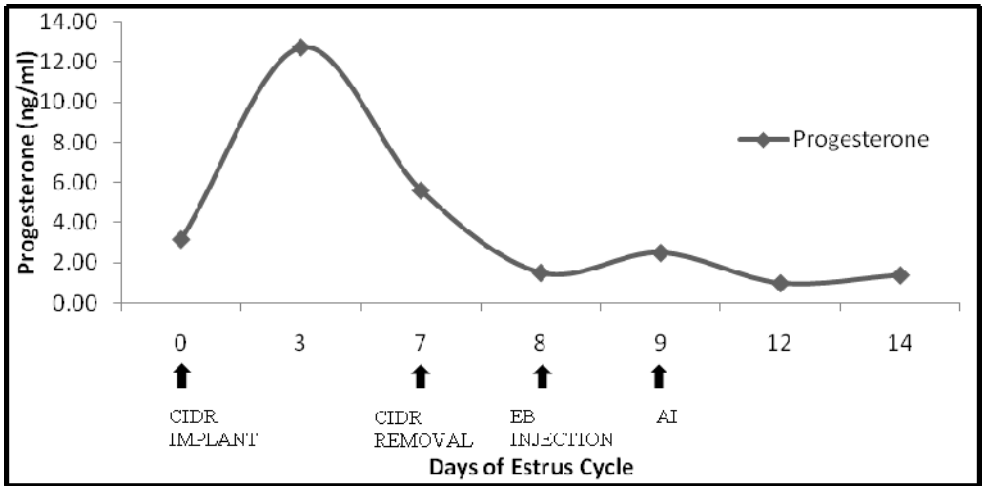


Figure 1. Progesterone profile in the peripheral plasma of synchronized cows

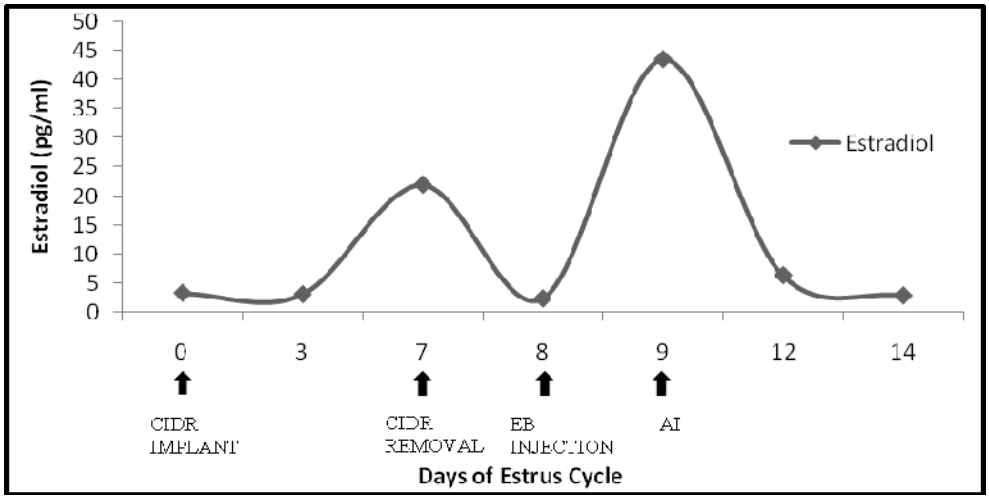


Figure 2. Estradiol profile in the peripheral plasma of synchronized cows

The result showed an increased concentration of progesterone when CIDR was inserted on day 0 and this started to decrease on day 3. This was due to the presence of natural hormone, progesterone, in the CIDR which was released once it was implanted into the vagina. Progesterone has a dominant role in regulating the estrus cycle. During diestrus with the functional corpus luteum, high concentration of progesterone inhibits the release of FSH and LH through its negative feedback control of the hypothalamus and anterior pituitary. High concentration of progesterone also inhibits behavioral estrus (2).

In the current study, probably estradiol benzoate injection triggers the release of estradiol from growing follicle which drastically increases LH secretion and within 24 hours, ovulation occurs.

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RAPID IDENTIFICATION OF SELEMBU SPECIES BY USING PCR-RFLP METHOD

S.M.N. Romaino[1*], Z.A. Fazly-Ann[1], S.S. Loo[1], M.M. Hafiz[1], M.I. Iswadi[1] and O. Abas-Mazni[2]

[1]Agro-Biotechnology Institute, Malaysia (ABI), P.O. Box 341, UPM Serdang, 43400 Serdang, Selangor, Malaysia; [2]Malaysian Agricultural Research and Development Institute (MARDI), Stesen MARDI Bukit Ridan, 26700 Pahang, Selangor, Malaysia.

*Email: romaino@mosti.gov.my

In early April 1983, Malaysia saw the birth of the first interspecies hybrid calves that were called Selembu. Nor Azman (1989) reported that Selembu showed the embodiment of vigor with huge body and sturdy limb and were found to be superior in growth performance at all life stages compared to their cattle parents. The animal posses black coat colour with brown patches of the forehead and along the midline.

Mitochondrial DNA (mtDNA) is a useful genetic marker as it is maternally inherited, and has very low recombination and fast evolution rate. Among the mtDNA genes, the Cytochrome b (*Cyt b*) gene was used in this study due to the sequence variability which makes it suitable for comparing species within the same genus or family. Thus, the objective in this study was to develop a rapid PCR-RFLP method for identification of Selembu species in Malaysia.

Blood samples were taken from five Selembu individuals at Pusat Ternakan Haiwan Lenggong, Perak, while 10 hair follicle samples of Selembu were collected at Ladang Jukimas, Sepang. Genomic DNA were extracted using QIAamp DNA Mini Kit and Qiagen Tissue Kit (QIAGEN GmBH, Hilden, Germany) according to the protocol provided by the manufacturer's handbook. The concentration of genomic DNA was then checked using Nanodrop (Thermo Scientific, USA). For polymerase Chain Reaction (PCR) amplification, one set Universal Primer was used to amplify the mitochondrial *Cyt b* gene. The PCR products were purified using Vivantis GF-1 PCR Clean Up Kit and submitted for DNA sequencing. The results of sequencing were then analyzed using BioEdit Version 7 followed by Restriction Mapper.

PCR amplification of mitochondrial *Cyt b* gene using Universal Primer produced a 486 bp fragment in all Selembu samples (Figure 1). The amplicons obtained after PCR reactions were subjected to digestion with restriction enzymes, namely *Xba*l, *Ssp*l and *Nla*lV (Table 1). Using the restriction enzymes *Xba*l, two fragments with a size 367 and 119 bp were detected, while *Ssp*l resulted in 382 and 104 bp fragments and *Nla*lV resulted in 343 and 143 bp fragments (Figure 2).

Based on the results, polymorphism obtained from cutting of the amplified product of locus *Cyt b* gene using Universal Primer suggests a reliable method of identification. Significant polymorphism obtained from a combination of PCR amplification followed by RFLP analysis, provides a suitable marker for the identification of Selembu. As a conclusion, all the samples collected in this study could be easily recognised using the Universal Primer and restriction enzyme *Xba*l, *Ssp*l and *Nla*lV.

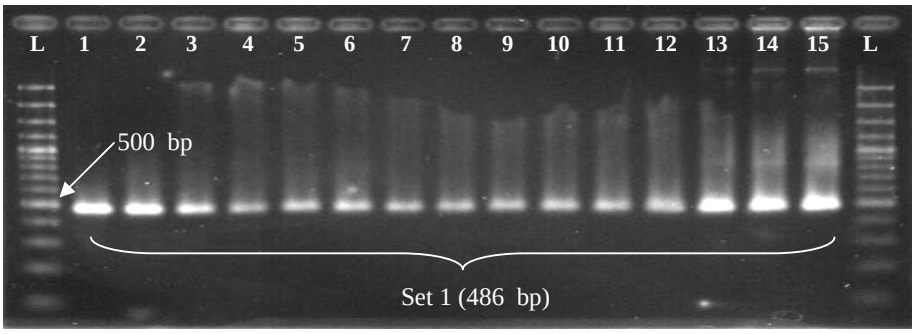


Figure 1. Polymerase Chain Reaction (PCR) amplification of mitochondrial *Cyt b* gene using primer Uni 1 produced a 486 bp fragment for Selembu species. L: 100 bp ladder

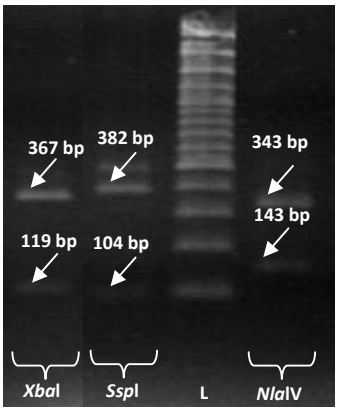


Figure 2. Restriction Fragment Length Polymorphism (RFLP) of mtDNA *Cyt b* gene. PCR amplicons using Universal Primer were subjected to restriction analysis with *Xba*I resulted in 367 and 119 bp fragments, *Ssp*I resulted in 382 and 104 bp fragments and *Nla*IV resulted in 343 and 143 bp fragments. L: 100 bp ladder

Table 1: Estimated fragment sizes after digestion of mitochondrial *Cyt b* gene in Selembu

Primer	Enzymes	Selembu
Universal Primer	*Xba*I	367 & 119 bp
	*Ssp*1	382 & 104 bp
	*Nla*IV	143 & 343 bp

This study was supported by R&D Initiative Fund under the project no. 06-05-ABI-AB003, Agro-Biotechnology Institute, Malaysia (ABI), Ministry of Science, Technology & Innovation.

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IDENTIFICATION OF FECUNDITY GENES IN MALIN AND DORPER SHEEP

W.M.Z. Somarny[1*], K. Aslinda[1], E.A.R. Roziatul[1], M.S.S. Ainu Husna[1] and M.O. Nurulhuda[2]

[1]Strategic Livestock Research Centre; [2]Muadzam Shah MARDI Station.
Malaysian Agricultural Research and Development Institute (MARDI), Serdang.
*Email: zsomarny@mardi.gov.my

Various major genes have been reported to affect prolificacy in sheep. These include three related oocytes-derived members, which are Growth Differentiation Factor 9 (GDF-9), Bone Morphogenic Protein 15 (BMP-15) and Bone Morphogenic Protein Receptor type 1B (BMPR-1B) (1). The BMPR-1B influenced the fecundity of Booroola Merino sheep (*FecB*) and is responsible for ovulation rate and little size (2, 3). The BMP-15 is important for ewe fertility (4) and consisted of four alleles, $FecX^H$, $FecX^I$, $FecX^B$, and $FecX^G$ (5). The point mutation on BMPR-1B and BMP-15 genes are expected to have an effect on ovulation rate in sheep. Therefore, the objective of this study was to identify if these fecundity genes are polymorphic, and to find if they are responsible for the prolificacy in Malin and Dorper sheep.

Blood sample was collected from 20 heads of Malin and Dorper sheep and genomic DNA was extraction from these blood samples using Promega Extraction kit (Promega, USA). Four genes related to fecundity, *FecB*, $FecX^I$, $FecX^H$ and $FecX^B$ (Table 1) were amplified by PCR. The PCR amplification was carried out in 25 µl reaction mixture including each primer (0.8 µl of a 10 µM solution). The PCR products were purified (Promega, USA) and sequenced (Applied Biosystems 3730xl DNA Analyser). The purified products were subjected to Forced Restricted Fragment Length Polymorphism using RE enzyme (Table 1) to detect mutation in *FecB* and *FecX* genes.

Table 1: Oligonucleotides, enzymes, and sizes of the genes before and after digestion

Mutation	Primer sequence (5'→ 3')	Product Size	RE	Digested products Size
FecB	CCAGAGGACAATAGCAAAGCAAA CAAGATGTTTTCATGCCTCATCAACAGGTC (6)	190 bp	*AvaII*	160 bp, 30 bp
$FecX^H$	TATTTCAATGACACTCAGAG GAGCAATGATCCAGTGATCCCA (7)	240 bp	*SpeI*	218 bp, 22 bp
$FecX^I$	GAAGTAACCAGTGTTCCCTCCACCCTTTTCT CATGATTGGGAGAATTGAGACC (4)	154 bp	*XbaI*	124 bp, 30 bp
$FecX^B$	GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA TTCTTGGGAAACCTGAGCTAGC (8)	153 bp	*DdeI*	122 bp, 31 bp

The *FecB* mutation in BMPR-1B gene and, $FecX^H$, $FecX^I$, $FecX^B$ alleles in BMP-15 were successfully amplified by PCR in all samples tested (Malin and Dorper sheep). In the case of *FecB* carriers (mutation), the individual has an *AvaII* restriction site (G/GACC) and for non-carrier lacked of this site. Products containing the *FecB* mutation were digested to yield a 160 bp fragment, whereas non-carrier remained uncut. These conditions are same in $FecX^H$ and $FecX^I$ alleles, the non-carriers remained uncleaved after digested with *SpeI* and *XbaI*, respectively (Table 1). However, for non-

carrier individuals with *FecX^B*, *DdeI* digested the products and produced 122 bp and 31 bp fragments. From these results showed that none of the samples carried the *FecB* mutation in BMP1R-1B gene, similarly no *FecX^H*, *FecX^I*, *FecX^B* mutations in BMP15 gene. All the individuals were wild homozygous. The homozygote for three BMP-15 alleles in ewes is infertile whereas the heterozygous individuals have a greater ovulation rate size (8). This study supports the previous study that noted the *FecB* and *FecX* mutations genes were not the only responsible causes to high prolificacy (3) as phenotypically Malin sheep are highly prolific. This finding is preliminary and therefore, further investigation of other genes that may be involved in prolificacy should be carried out as well as good breeding record is required to know the association of the genes with prolificacy trait.

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PHYLOGENETIC RELATIONSHIPS OF THREE CATTLE BREEDS IN MALAYSIA

K. Aslinda[1,4]*, W.M.Z. Wan Somarny[1], S. Md Tasol[1], I. Fadzlirahimi[2], A.W. Mohd. Hafiz[3] and M.N. Siti Azizah[4, 5]

[1]Strategic Livestock Research Centre, MARDI Headquarters, 43000 Serdang; [2]Kemaman MARDI Station, P.O.Box 44, 24007 Kemaman, Terengganu.; [3]Muadzam Sabah MARDI Station, 26700 Muadzam Shah, Pahang ; [4]School of Biological Sciences, Universiti Sains Malaysia, Penang and. [5]Centre for Marine and Coastal Studies, Universiti Sains Malaysia, 11800 Minden, Penang
*Email: linda_3685@yahoo.com

Domestic cattle breeds are mostly grouped into two closely related species; *Bos taurus* and *B. indicus*, both descending from *B. primigenius* (2). Mitochondrial DNA (mtDNA) are widely used in phylogenetic studies because of several advantageous characteristics it possesses; maternal inheritance, no recombination and fast rate of evolution (1) permits investigation of recent history and serves as a good genetic marker for population studies. The purpose of this study was to elucidate the phylogenetic relationships of three cattle breeds; Kedah-Kelantan (KK), Yellow Cattle (YC) and Brahman using mtDNA ND5 sequence. The KK cattle are an indigenous breed in Malaysia while the Yellow Cattle (YC) and Brahman (Brah) are indigenous to China and India. These breeds are categorized in the *B. indicus* lineage.

Genomic DNA extracted from blood samples of YC (n=16), Brahman (n=3), KKB (n=28, Pahang) and KKC (n=26, Terengganu) using Genomic DNA Purification Kit (Promega, USA) was amplified using primers for cattle mtDNA ND5 gene fragment (F-5'CCCAACGAGGAAAATATACC3', R-5'GGAAGAGGTTGTTTGC GGTT3') which generated a 755bp fragment (3). Amplification was conducted in a Bio-Rad C1000TM Thermal Cycler with 40 cycles of denaturation (94°C, 30s), annealing (62°C, 30s) and extension (72°C, 35s), followed by a final extension of 72°C for 10min. The PCR products were purified using the PCR Clean-Up System Kit (Promega,USA) and sequenced. All sequences were checked and aligned using MEGA ver.4.0 (4) while confidence limits were calculated using bootstrap with 1000 replicates for Neighbour Joining method generated by Kimura two-parameter model**.**

Table 1: Nucleotide composition of ND5 partial sequence of three cattle breeds.

Nucleotide	Average %	1st codon position %	2nd codon position %	3rd codon position %
T	31.3	29.0	41.3	23.6
C	24.8	18.4	24.5	31.5
A	35.2	41.5	26.2	38.0
G	8.6	11.2	7.9	6.9

Final alignment after editing using MEGA ver4.0 (4) was 696 bp sequences. The 10 haplotypes generated were compared with reference bovine mtDNA sequence, GI: 584939 (3). A total of 22 variables sites (3.16%) with 8 parsimony informative and 14 of singletons were observed. There was no insertion/deletion. The average nucleotide frequencies were as in Table 1. The nucleotide composition was richer in A/T nucleotide (66.5%) than C/G (33.4%). The third position showed an imbalance of base usage with infrequent G (6.9%) and biased towards A+C (69.5%). The low number of G's and high number of A's (38.0%) at the third position showed that the likelihood of an A to G transition is lower than a G to A transition (5).

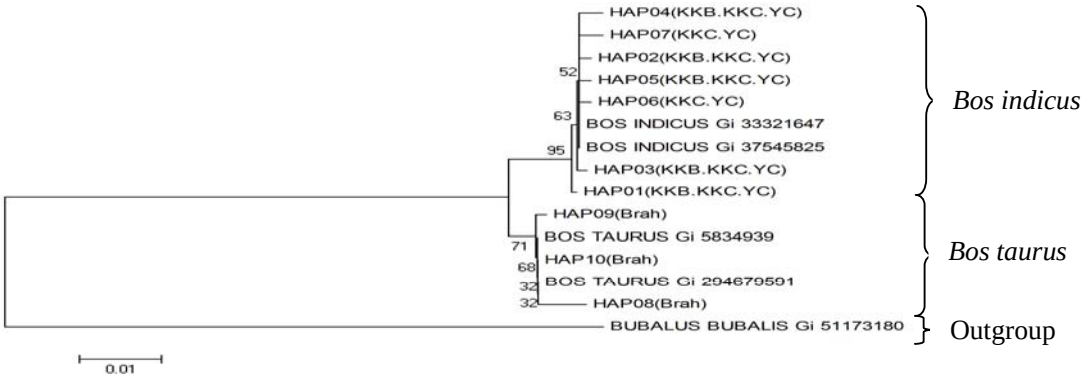


Figure 1: Neighbour joining tree of ND5 partial sequence of 10 haplotypes.

The NJ phylogenetic tree (Figure 1) was constructed with the 10 haplotypes and an additional five sequences cited in GenBank with bootstrap confidence of 1000 replicates. Two distinct lineages were observed depicting the *B. indicus* and *B. taurus* maternal origins. The KK and YC breeds clustered into the *B. indicus* lineage with high bootstrap probability (95%). Interestingly, three haplotypes (Hap08-Hap10) of Brahman cattle clustered into the *B. taurus* lineage with a bootstrap probability of more than 50%. This Brahman was imported from Australia and was developed with some infusion of British breed. Thus, its taurine gene was from the female cattle while the zebu gene came from the male line (6). Further studies in paternity area with large number of Brahman are necessary to verified these finding.

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CONSERVATION OF *ACACIA MANGIUM* PLANT ON TANNIN CONTENT

M. Nurzillah[1*], H. Wan Syahidah[2], S.A. Syed Hussein[3] H.Haryani[1] and S. Shariff[1]
[1]Veterinary Institute Malaysia, KM 13, Jalan Batu Pahat, Beg Berkunci 520, 86009 Kluang, Johor;
[2]Veterinary Public Health Laboratory, Department of Veterinary Services, Bandar Baru Salak Tinggi, 43900 Sepang Selangor[3]; Department of Veterinary Services, Ministry of Agriculture and Agrobased Industries, Wisma Tani, Blok Podium, Lot 4G1, Presint 4, Putrajaya,
*Email: nurzillah@dvs.gov.my / nurzillah@yahoo.com

Acacia mangium is a fast growing leguminous plant use in plantation forestry programme. It plays the role of sustaining commercial supply of tree products mainly timber. Acacia plant can be used as animal feed, but its level of inclusion in the feed is quite limited due to the presence of anti-nutritional factors (1). The main compound that has been identified is tannin (2). Tannins can bind with macromolecules (proteins, structural carbohydrates and starch) and decrease their availability to digestion. They are divided into two major groups, hydrolyzable and condensed tannins. *Acacia mangium* are represented by the hydrolysable tannin. Tannins have been shown to affect palatability and can be caused ill effects if given at high dosage (1, 2). Tannins have been shown to affect the microflora in the rumen and thus the efficiency of digestion (3, 4, 5). Acacia plants (*Acacia mangium*) are found abundantly in Malaysia and around the National Feedlot Centre in Gemas. The growth is so extensive that Acacia is regarded as weed in fodder growing areas. Acacia seeds are found in abundant in the soil as such that its growth is difficult to control. The objectives of the study was to determine the levels of tannins in Acacia plants at different ages and the effects of denaturing of tannin through fermentation and treatment by ensiling young Acacia plants.

In the trial, young Acacia plants were harvested, the main stems were removed and the forage was wilted for 4 hours. It was then chopped and ensiled in plastic barrel. The experimental treatments were as follows: Acacia plants (control), Acacia plants + microbe, Acacia plants + urea + microbe, Acacia plants + molasses + urea + microbe. In the trial, after the first cutting, the Acacia plants were allowed to re-grow and samples of Acacia plants were harvested at different ages (28, 35 and 42 days) to determine the tannin content. The harvested samples were dried and stored and analyses for tannin content.

Spectrophotometric method was used to determine tannin in plants because of its sensitivity, specificity and simplicity (6). All Acacia samples were extracted using solvent extraction and determined based on the KIO_3 reaction before the measurement of colored product at 550nm according to modified method of Carla et al. (7).

The results of conserving Acacia plants are shown in Table 1. Fresh Acacia plants contained 1.7% tannin. Ensiling the Acacia plants reduced the tannin content. Further reduction in tannin content was noted with different treatment parameters. The addition of urea, molasses and microbes saw the level of Acacia to be the lowest. According to Makkar (5), addition of urea and then storage (urea-ammoniation) increased the extent of tannin inactivation. Inactivation of tannins during storage was due to their polymerisation to higher 'inert' polymers. Table 2 shows the yield of Acacia after the first harvest. The yield of Acacia petiole (leaves) was very low. Re-growth was slow and this was much affected by other environmental conditions. Overall, the amount of tannin in Acacia plants seemed to reduce with maturity. As much as 28% of tannin content was reduced from 35 days to 63 days of maturity of the plant.

Table 1: The effect of ensiling on tannin content of *Acacia mangium*

Treatment Silage	Tannin content (%)
Acacia (control)	1.49
Acacia + microbe	1.21
Acacia + urea + microbe	1.13
Acacia + urea + molasses + microbe	0.96
Acacia (fresh)	1.68

Table 2: Yield and tannin content of *Acacia mangium* at different stage of maturity.

Age of Acacia (days)	Average yield (MT/ha)	Tannin content (%)
35	1.25	1.47
42	1.60	1.38
63	2.05	1.06

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FUNGAL FERMENTATION OF CASSAVA AND ITS EFFECT ON THE *IN VITRO* DEGRADATION RATE

M. Ramin, H. Yaakub*, A.R. Alimon and Z.A. Jelan
Department of Animal Science, Faculty of Agriculture, University of Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
*Email: hali@agri.upm.edu.my

Cassava is a major root crop in tropical countries, it is currently a worldwide subject to develop its potential as feed for animals especially ruminants. Cassava is very low in protein, but contains high amount of starch (3). Because of the low protein of cassava, their use in animal feeding requires supplementation of such diets. Traditionally, foods of animal origin are relied upon to meet the protein needs. However, conventional protein sources (fish, meat and egg) are expensive. Protein enrichment of cassava by using methods which are less expensive is desirable. As solid state fermentation (SSF) and enzyme treatments are potentially beneficial. Fungal fermentation has been recognized to be an inexpensive method for increasing the protein level under SSF. As *in vivo* experiments might not be the most suitable technique for evaluation of feed treatment benefits (1), an *in-vitro* gas production technique was utilized in the present experiment. The objective of this experiment was to estimate the increase of the nutrient value of cassava by the SSF and consequently determine its degradability rate by using *in-vitro* gas production technique.

Tapioca was fermented for 10 days under solid state fermentation culture with two different fungi (*Aspergillus niger* and *Rhizopus oryzae*). Rumen contents were collected in pre-warmed flasks 2h before morning feeding from three fistulated goats fed a normal diet (60% hay and 40% concentrate). Rumen fluid was obtained by squeezing rumen contents through four layers of cheese cloth. The strained rumen fluid was mixed in the ratio of 1:2 (v/v) with anaerobic medium (buffer) to form suspension (2).

A 3×3 factorial design was performed and analyzed by the General Linear Model (GLM) procedure, using a statistical package of SAS version 9.2 (4). The model used was:

$$Y_{ijr}=\mu+t_i+d_j+ (td)_{ij}+e_{ijr}$$

Where Y_{ijr} is the dependent variable, μ is the overall mean, t_i is the effect of treatment, d_j is the effect of day, $(td)_{ij}$ is the interaction of day and treatment and e_{ijr} is the residual error. The means were compared by Duncan multiple range test and considered significant at $P< 0.05$.

There was an increase ($P<0.05$) in crude protein concentration due to treatment of cassava with *A. niger* and *R. oryzae* compared to the unfermented treatment (fresh cassava). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were greater ($P<0.05$) for both fermented treatments compared to the unfermented treatment (Table 1). A follow-up *in-vitro* gas production experiment was conducted in order to determine the kinetic rate of fermentation (degradation rate) using fermented cassava. The rate of fermentation, *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) were greater from the cassava fermented with *R. oryzae* after 10 days SSF compared to cassava fermented for 10 days with *A. niger* or the unfermented cassava. However, total gas production from the treatment fermented with *A. niger* for 10 days under SSF was lower compared to the treatment fermented with *R. oryzae* after 10 days or the unfermented one (Table 2). It was concluded that *A.*

niger and *R. oryzae* are effective fungi for treatment to increase the CP of cassava suppressing gas production *in vitro*. However, the degradability rate of cassava fermented with *R. oryzae* was greater compared to the fresh cassava. Further experiments are needed to demonstrate their potential use as an inexpensive technique for increasing the nutritional value of cassava.

Table 1. Effects of different treatments, days and their interaction on crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose (HC) and ash concentrations under solid state fermentation.

Parameter	Treatment Mean (±SE)			Contrasts (P <)		
	A	B	C	treat	day	treat*day
CP	11.2±0.93[a]	19.7±1.72[b]	12.9±0.2[c]	<.0001	0.01	<.0001
NDF	29±2.02[b]	39.4±2.5[a]	39±2.12[a]	0.0003	0.02	0.01
ADF	2.3±0.17[b]	7.3±1.1[a]	2.8±0.1[b]	<.0001	<.0001	<.0001
HC	27±2.13[b]	32±2.4[a]	36.2±2.08[a]	0.003	0.181	0.01
Ash	39±2.68[a]	74.3±11.5[b]	36±2.3[a]	<.0001	0.052	0.006

Means within a row with different letter(s) differ significantly (P < 0.05).
A: Control (mean of 4, 7 and 10 days fermentation): B: *A. niger* (mean of 4, 7 and 10 days fermentation).
C: R. oryzae (mean of 4, 7 and 10 days fermentation).
± SEM

Table 2. The gas production kinetics from fresh cassava and from cassava originating from a 10-day fungal cultivation with *Aspergillus niger* or 10-day cultivation with *Rhizopus oryzae.*

	a	b	c (h^{-1})	Total gas 48 h (ml)	ME (MJ/Kg DM)	IVOMD (%)
Cassava–fresh (control)	-0.29	63.01	0.05	59	8	58.0
Cassava – *A. niger*	6.79	37.02	0.02	26	6.07	46.0
Cassava – *R. oryzae*	-7.05	57.84	0.13	60	9.26	66.3

c = rate of fermentation

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NUTRITIVE VALUE OF CORN SILAGE ENSILED IN PLASTIC BAG

H. Wan Syahidah[1*], M. Helen[2], M.S. Lily Suhaida[1], G. Ernah[1] and M. Fuziah[2]
[1]Veterinary Public Health Laboratory, Department of Veterinary Services,
Bandar Baru Salak Tinggi, 43900 Sepang Selangor & [2]Department of Veterinary Services, Ministry of Agriculture and Agrobased Industries, Wisma Tani, Blok Podium, Lot 4G1, Presint 4, Putrajaya
*Email: syahidah@dvs.gov.my

Maize silage has been the basic fodder for cattle in many countries. The high starch content of the grain makes the energy content of maize higher than that of hay or forage sorghum and thus is good material for silage production (1). Many available tropical forages and agro-by-products are generally low in nutritive quality. Silage made from these forages not able to sustain high animal productivity because of their low digestible energy content. New methods of silage making may be needed to improve the nutritive values of low quality forages. Large scale silage making involves the use of silos and containers. It is worthwhile to use a more practical container in terms of versatility and cheap in small scale silage production. This study was undertaken to evaluate the nutritive values of corn silage with added ingredients and using disposable type viz plastic material as the container.

The trial was divided into 2 groups of silage which consisted of post harvest corn stalk without any corn cob and corn stalk with at least one corn cob. Each group was divided into 4 treatments which are the control silage using only corn (T1), mixture of corn added with 4% molasses (T2), mixture of corn added with 4% effective microorganisms (T3) and mixture of corn added with 2% molasses and 2% effective microorganisms (T4). The corn plants were cut into ½ to ¾ inch before mixed and packed. Each treatment was packed in 20kg double layered plastics bags for the first layer and gunny sack as the outer layer. In the trial, after the treatment, the silages were opened at 21 days to determine the nutritive values. Each sample was oven dried at 60°C for 24 hours before laboratory proximate analyses. The proximate analyses were carried out according to AOAC (3). The calcium and phosphorus contents were determined using Atomic Absorption Spectrometry-flame absorption and UV Spectrometry according to AOAC (3).

Nutritive values of corn silage are as shown in Table 1. They are significantly different in proximate composition at $p<0.05$ in both groups. The range of dry matter for both groups is 20-25%. The lowest dry matter is in treatment 2 (group 1), 20.21% while the highest is in treatment 3 (group 1), 24.96%. The amount of crude protein is not significantly different in group 1 (range 9-10%) but is significantly different in group 2. In group 2, the highest amount of crude protein is 13.52% (treatment 2) and the lowest is in treatment 1 (11.38%). According to Kung et al (4) corn silage is usually combined with other feeds, such as alfalfa silage, soybean meal, and fishmeal to increase crude protein concentrations. The metabolizable energy and TDN values showed significant difference in treatment 3 and 4 in both groups despite the addition of molasses. The range of crude fat, crude fibre and total ash in both groups are 1.2-3.2%, 24.6-29.3% and 11.6-28.8% respectively. The highest amount of crude fat, crude fibre and total ash are in treatment 2 (group 2) 3.23%, treatment 1 (group 1) 29.33% and treatment 4 (group 1) 28.88% respectively. The calcium and phosphorus content are not significantly different ($p>0.05$) in both group. The ranges of calcium and phosphorus contents are 0.6-1.0% and 0.7-1.2%, respectively.

Table 1 Nutritive value of corn silage using plastic bag method

	Silage Treatment (mean± SD)			
	T1	T2	T3	T4
Group 1				
DM (%)	20.21±1.68^{b}	23.65±1.05^{a}	24.96±1.38^{a}	24.29±3.29^{a}
CP(%)	10.85±4.28^{a}	10.48±3.55^{a}	9.91±3.65^{a}	9.63±3.00^{a}
EE (%)	1.71±0.57^{ab}	1.79±0.21^{a}	1.25±0.32^{c}	1.52±0.38^{b}
CF (%)	29.33±4.86^{a}	28.91±4.43^{a}	27.23±5.96^{ab}	24.63±5.47^{b}
Ash (%)	17.32±5.52^{c}	16.05±1.99^{c}	22.76±4.92^{b}	28.88±3.75^{a}
Calcium (%)	0.76±0.96 ^{a}	0.76±0.99^{a}	1.04±1.03^{a}	0.83±1.04^{a}
Phosphorus (%)	1.01±0.62^{a}	0.70±0.41^{a}	0.85±0.43^{a}	0.83±0.42^{a}
TDN (%)	51.87±4.57^{a}	52.69±1.5^{a}	47.58±6.31^{b}	42.76±3.76^{c}
ME (MJ/kg)	7.65±0.75 ^{a}	7.78±0.25^{a}	6.94±1.03^{b}	6.15±0.62^{c}
Group 2				
DM (%)	21.71±1.5^{a}	20.61±0.91^{b}	21.35±0.66^{a}	21.58±0.56^{a}
CP (%)	11.38±2.7^{b}	13.52±2.03^{a}	12.96±3.25^{ab}	13.00±2.45^{ab}
EE (%)	2.93±0.73^{a}	3.23±0.47^{a}	3.01±0.37^{a}	3.09±0.51^{a}
CF (%)	25.02±3.75^{b}	28.90±3.91^{a}	26.95±2.81^{ab}	28.94±4.16^{a}
Ash (%)	12.68±4.37^{ab}	11.66±1.55^{b}	14.16±2.05^{a}	13.60±1.28^{a}
Calcium (%)	0.66±1.03^{a}	0.72±1.04^{a}	0.96±1.11^{a}	0.92±1.06^{a}
Phosphorus (%)	1.16±0.57^{a}	1.25±0.55^{a}	1.24±0.53^{a}	1.20±0.44^{a}
TDN (%)	56.98±3.80^{a}	56.38±1.51^{a}	54.53±1.21^{b}	54.87±1.10^{b}
ME (MJ/kg)	8.49±0.63^{a}	8.39±0.25^{a}	8.08±0.20^{b}	8.14±0.18^{b}

Means with a different superscript in each row are significantly different ($p<0.05$).
Where DM=dry matter, CP=crude protein, EE=ether extract, CF=crude fibre, TDN=total digestable nutrient. ME= metabolizable energy

Overall, group 2 presents better nutritive value than group 1 with higher amount of crude protein and metabolized energy. Silage made by corn stover with at least one corn cob can be one of alternative ways of increasing the nutritive value. Plastics silage bags are an economical alternative to traditional silage storage systems, such as pits and silos. It is also an effective way for preserving feed with minimum nutrient loss. The anaerobic environment that is created eliminates spoilage from the growth of yeasts, molds and adverse bacteria while maintaining essential proteins and nutrients.

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NUTRITIVE VALUE OF *LABLAB PURPUREUS*, A POTENTIAL RUMINANT FEED

B. Mohd Saufi[1], S. Noraini[2], M. Mardhati[2], Rosnizah[2], A. Dzulfazly[1], S. Baharin[1] and S. Shanmugavelu[2],

[1]Strategic Livestock Research Center, Stesen MARDI Kluang, Kluang Johor.
[2]Strategic Livestock Research Center, MARDI.Serdang Selangor
Email: msaufi@mardi.gov.my

Lablab (*Lablab purpureus*) is commonly grown in Indonesia, China, Japan, West Africa and South East Asia. It is also popular as a nitrogen-fixing green manure to contribute to soil N and improve soil quality and has been used as rotational crop to increase soil quality. *Lablab* is a climbing erect perennial herbaceous crop often grown as an annual, however the *Lablab* planted in Kluang has shown non-seasonality growth without flowering or fruiting. *Lablab* was reported contain high protein (15-30%) with high lysine digestibility (55%) (1). Observation and evaluations of *Lablab* forages planted in Stesen MARDI Kluang has shown this forage is able to grow well either in polybags or in the ground. This paper reports on the proximate analysis and amino acid content of *Lablab* grown plant.

Briefly, *Lablab* shoot, leaf and young stem with leaf were subjected to proximate analyses in triplicates according to AOAC (2). Fiber analyses (NDF, ADF and ADL) were carried out using Filtering Bag Technique (ANKOM Technology Method). The concentration of hemicelluloses was calculated as the difference between NDF and ADF while that of cellulose as the difference between ADF and acid detergent lignin (ADL). Amino acids content of the dried *Lablab* was determined using amino acid analyzer (Biochrom 30) based on established methodology (3).

The proximate analysis and amino acid profiles of ground oven dried *Lablab* is shown in Table 1 and 2 respectively. Dried shoots stem and leaves of *Lablab* contained of 89.9, 91.2 and 89.3% of dry matter and 17.2, 9.3 and 35.4% crude protein respectively. Results have indicated that dried lablab contained low fat and ash. Shoot, stem and leaves contained 7.0, 6.0 and 7.7 % ash and 0.6, 2.3 and 3.0 fats respectively. While amino acid content of shoots and leaves was 39.92 and 55.10 % of total crude protein. These values were quite similar to those reported by Mapiye *et al* (4).

Table 1. Chemical composition of oven dried *Lablab*

Part of plant	DM	ASH	CP	N	EE	GE	CF	ADF	ADL
Shoot	89.9	7.0	17.2	2.8	0.6	16.4	72.7	47.0	0.5
Rod	91.2	6.0	9.3	1.5	2.3	18.6	66.1	47.2	2.5
Leaves	89.3	7.7	35.4	5.7	3.0	17.9	69.8	28.7	0.9

Data based % dry matter basis; DM: dry matter; CP: crude protein; N: Nitrogen; EE: Ether Extract (fat); GE: gross energy; CF: crude fiber; ADF:Acid Detergent Fibre; ADL:Acid Detergent Lignin

The use of *Lablab* as ruminant feed was reported to reduce feed consumption in beef cattle while its protein content was adequate for growing heifers (5, 6). However, local feeding trials are necessary to evaluate its nutritive value for Malaysian cattle. *Lablab* was also reported contain no/minimal anti-nutritional factor as compared to gliricidia (5). *Lablab purpureus* with its enhanced nutritive value is a fodder crop of great significance for the tropics. *Lablab* can be used advantageously as a cover crop to

provide organic matter, minerals and fix nitrogen into the soil thereby improving crop yield in an economic and environmentally friendly manner. Based on its composition, *Lablab* is a potential ruminant feed in Malaysia and should be investigated further. This plant can become another important feed resource for ruminant farming in Malaysia.

Table 2. Amino acid content (%) of *Lablab*

EAA	Leaves	Shoots
Methionine	0.05	0.05
Lysine	0.73	0.45
Leucine	1.03	0.67
Histidine	0.37	0.21
Phenylalanine	0.89	0.54
Valine	0.67	0.51
Arginine	0.73	0.37
Threonine	0.90	0.53
Tryptophan	0.12	0.06
Isoleucine	0.65	0.42
Aspartic Acid	1.58	1.51
Serine	0.83	0.57
Glutamic Acid	0.94	0.69
Glycine	1.62	1.07
Alanine	1.49	0.94
Cystine	0.02	0.01
Tyrosine	0.64	0.39
Proline	0.85	0.49
Total (EAA + NEAA)	14.15	9.48
Non Protein	21.26	7.73
Total AA as % of CP (%)	39.92	55.10

EAA:Essential Amino Acid;NEAA:Non-Essential Amino Acid; CP: Crude Protein

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PROXIMATE AND MINERALS COMPOSITION OF *Acacia mangium*: CULTIVATED *vs* WILD

A. M., Marini [1*], S., Shanmugavelu[2], I., Fadzlirahimi[1], , M., Abdul Razak[1], I., Mazwan[1], M.S., Siti Masidayu[2], P., Awis[1] and B., Mohd Firdaus[1]

[1]Strategic Livestock Research Centre, Kemaman MARDI Station, P.O. Box 44, 24007 Kemaman, Terengganu, [2]Strategic Livestock Research Centre, MARDI HQ, G.P.O. Box 12301, 50774 Kuala Lumpur.
E-mail: marini@mardi.gov.my

Protein-rich foliages are an important feed resource for ruminants in all tropical countries, especially in the dry season when green grasses are normally scarce. Their utilization as a supplement can improve voluntary feed intake, digestibility and animal performance. There are abundant of *Acacia mangium* in Malaysia and the favourable characters of the species are ease of establishment, wide environmental tolerance, fast growth rate and ability to produce large quantities of biomass. Despite these favourable agronomic characteristics and its wide utilization as forage in some regions, the low dry matter digestibility (DMD) and high tannin levels of *Acacia* sp. generally make it inadequate as the sole source of nutrition for ruminants (1). The *Acacia* sp. is rich in crude protein (CP) and minerals and therefore it can be a useful supplement for forage based diets with low CP. This paper reports the proximate and mineral composition of cultivated *A. mangium* leaves compared to those obtained from the wild.

In this study, *A. mangium* were planted in Kemaman MARDI Station using seeds from Forest Research Institute Malaysia (FRIM). The *Acacia* seeds were scarified with hot water for 5 min before sowing in a tray. After three weeks, the plants were transferred to polybags and then transplanted to the field plot after 4 months. A total of 300 plants per plot were sowed with a distance of 1 m between rows and 0.5 m between plants. At age of six months or plant height between 1 to 2 m, the plants were harvested and separated into leaves and stems. Leaves of *A. mangium* from wild populations about the same height were collected in Kemaman MARDI Station. All samples were oven dried at 50°C for 24 hours. The samples were then ground to pass through a 1 mm sieve prior to chemical analysis in triplicates. Proximate analyses of samples were determined according to standard methods for CP determination of, moisture content, ash and crude fat (2). Fibre analyses were carried out according to the method described by Van Soest (3). The concentration of hemicelluloses was calculated as the difference between NDF and ADF. Mineral concentrations were determined using atomic absorption spectrophotometer except for the phosphorus which was determined using UV-VIS spectrophotometer.

Proximate and mineral compositions were calculated based on DM basis as shown in Table 1. Results indicate that the CP content of the cultivated *A. mangium* leaves was higher (17.42%) than the wild plants (14.95%). There was a general improvement in the nutritive value of the cultivated *A. mangium* leaf compared to the wild plants in fibre analysis (except for hemicellulose value). NDF and ADF levels in leaves of the cultivated plants were reduced from 47.73% to 45.90% and 36.89% to 28.65%, respectively. Only a few minerals were higher in the cultivated *A. mangium* leaves compared to the wild type.

In this study, a substantial variation in feed quality was observed between the cultivated and wild populations of *A. mangium*. The CP and fibre content of forages is influenced by genetics, stage of growth and environmental conditions while its mineral content by the soil mineral condition (4). It is interesting to note that the CP content of

A. mangium was improved under cultivation compared to the wild by almost 2.5%, probably due to decrease in ADF. Another interesting observation is a 50% decrease in the copper content of the cultivated *A. mangium* compared to the wild. This is an important finding as *A. mangium* has been commonly utilized as a bio-remedial plant to reclaim land with elevated metal concentrations (5). It is therefore essential to determine the elemental status of the soil prior to planting to prevent excessive uptake of minerals that can be detrimental if utilised as livestock feed. The data from the present study shows the potential of cultivating *A. mangium* as animal feed based on its nutrient composition and at the same time highlights precautions necessary to prevent excessive accumulation of minerals.

Table 1. Proximate and mineral composition (% dry matter basis) in leaves of cultivated *A. mangium* compared to wild populations

Nutrient composition (% dry matter basis)	*A. mangium* leaves	
	Cultivated	Wild
Proximate		
Crude protein (CP)	17.42 ± 0.97	14.95 ± 0.14
Ash	4.28 ± 0.17	3.91 ± 0.19
Crude fat	2.98 ± 0.31	4.35 ± 0.08
Moisture content	6.02 ± 0.76	8.34 ± 0.06
Dry matter	93.98 ± 0.76	91.67 ± 0.06
Crude fiber	27.84 ± 6.67	30.99 ± 0.56
Neutral detergent fiber (NDF)	45.90 ± 1.44	47.73 ± 0.96
Acid detergent fiber (ADF)	28.65 ± 0.84	36.89 ± 1.01
Nitrogen free extract (NFE)	41.48 ± 8.26	37.47 ± 0.09
Hemicellulose	17.25 ± 0.66	10.85 ± 1.97
Mineral		
Calcium (%)	0.39 ± 0.09	0.67 ± 0.04
Copper (ppm)	5.76 ± 1.12	11.95 ± 0.52
Iron (ppm)	160.67 ± 64.23	115.49 ± 24.83
Potassium (%)	1.19 ± 0.14	0.86 ± 0.01
Magnesium (%)	0.15 ± 0.01	0.17 ± 0.01
Phosphorus (%)	0.13 ± 0.01	0.07 ± 0.01
Sulfur (%)	0.24 ± 0.02	0.20 ± 0.01
Zinc (ppm)	20.83 ± 5.90	27.22 ± 0.16

Data are presented as mean values ± SD (n=3)

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IN-SACCO DIGESTIBILITY OF HERB ADDED CONCENTRATE PELLETS AS RUMINANT FEED

I. Noraida*, N.A. Nik Fatihah and S. Meriksa
Institue of Biological Science, Faculty of Science, University of Malaya,
50603 Kuala Lumpur, Malaysia.
*Email: aida@um.edu.my

Ruminant animals are able to use fiber primarily composed of cellulose and xylan as an energy source because of a symbiotic relationship with microbes in the rumen. The rumen microbes produce a wide range of highly active plant cell-wall degrading enzymes and their contribution to fiber digestion is estimated to be 80% of total activity (1,2). Poor digestion of feeds and the cost of production with limited resource have become the problem of the small scale farmers in Malaysia (3). This non-conventional feeds need to be effectively utilized to achieve good performance in animal as well as being cost effective. Feeding ruminant with grass alone is usually not adequate to meet the nutrient requirement for this animal due to the variability of the quality of grass given, their relatively low digestibility, restriction on energy intake imposed by their bulk and system of feeding. Commercial pellets are suitable for feeding to ruminant as supplementary feed in times of poor pasture quality or in drought situations. Pellets as supplement in ruminant diet can increase gains and increase feed efficiency. The local herbs have been found to be effective against helminthes. Therefore, adding herbs into pellet should help to improve the rumen environment and increase digestibility of feeds in ruminant digestive system thus might increase the rate of digestion and the absorption of nutrients to support growth. In turn, an increase in digestibility will lead to increased in body weight, muscle mass and total body fat. The present study was to measure and compare the rate of digestibility of pellets in goat's rumen when added with local and imported herbs using the nylon bag technique.

One year old goat was fitted with rumen fistula to allow nylon bags to be introduced into the rumen. The nylon bags, 6 x 15 cm in size with a pore size of approximately 50 µm (Ankom Co., Fairport, NY) were used in series of *in sacco* ruminal incubations. Each nylon bag was oven-dried for 24 hours, weighed, filled with 2.0 gm of each treatment, heat sealed and tied with nylon string. Nine treatments used were 1) Commercial Pellet + imported herb (CPI) 2) Commercial Pellet + local herb (CPL), 3) Commercial Pellet + imported herb and Napier grass (CPIN), 4) Commercial Pellet + local herb and Napier grass(CPLN), 5) PKE Pellet + imported herb (PKEI), 6) PKE Pellet + local herb (PKEL), 7) PKE Pellet + imported herb and Napier grass (PKEIN), 8) PKE Pellet + local herb and Napier grass (PKELN), and 9) Napier Grass (N). There were four replicates for each treatment. The nine treatments were inserted into the goat's rumen and incubated for 24, 48, 72 and 96 hours respectively. Once taken out from the rumen, the bags were immediately submerged into cool water bath to stop microbial activity and then washed, oven-dried at 70°C for 24 hours, cooled at room temperature and weighed again. The same method was repeated for the 48, 72, and 96 hours treatments. The digestibility of the treatments was measured by calculating the percentages of the differences between the pre-incubation and post-incubation dry matter contents.

It has been expected that herbs added in ruminant diet support the growth and increases the rate of digestion in the ruminants. The result showed that the highest digestibility was obtained for the commercial pellet + local herb, with values of 68.81 ±

3.25% (24 hours), 76.57±2.18% (48 hours), 80.27±0.85% (72 hours) and 82.95±0.56% (96 hours) respectively. The lowest digestibility was for the Napier grass. The rate of digestion in all treatments increased when herbs were added into the mixture. The local herb was the most efficient in increasing the digestibility *in sacco.* This is expected to increase the absorption of nutrient to support growth and gain in body weight, muscle mass, total body fat and thus improve the carcass quality of the animals. The commercial pellet added with local herbs was more digestible than PKE pellet added with local herbs. This was probably due to the presence of palm kernel expeller in the PKE pellet. These products are high in fibre but low palatability, deficient in several amino acids, and low in lysine availability.

Addition of the local herbs in either commercial or PKE pellets increases the in sacco digestibility in the goat's rumen while adding napier grass to both mixtures of pellets and herbs slightly decreased the digestibility. Studies to further investigate the effects of different types and quantity of herbs on the weight gain and reproductive performances of the ruminants are still ongoing.

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IMPROVING THE NUTRITIVE VALUE OF RICE STRAW THROUGH SOLID STATE FERMENTATION USING THREE MICRO-FILAMENTOUS FUNGI

Most. Ferdousi Begum[1] and A.R. Alimon[1, 2]*
[1]Laboratory of Animal Production, Institute of Tropical Agriculture, [2]Department of Animal Science, Faculty of Agriculture, University Putra Malaysia, 43300 Serdang, Selangor, Malaysia.
* E-mail: ralimon@agri.upm.edu.my

The major limitations of straw as an animal feed are its low digestibility and low protein (CP) content. It is reported that microbial treatments including fungal, can improved digestibility of straw and its nitrogen content (1) and also produce large amount of cells rich in proteins, vitamins and minerals (2). However, the most suitable microorganisms and the condition of fermentation have not been established. The objective of this study was to compare the nutritive value of rice straw (RS) that was fermented under solid state (SSF) for seven days with three cellulolytic fungi (*Aspergillus niger*, *Trichoderma harzianum* and *Rhizopus oligosporus*) and addition of either urea, molasses or palm kernel cake (PKC). A 10% spore suspension of different fungi with 1×10^6 cell/ml was used to inoculate 15g substrate with 60% initial moisture content. Proximate analyses (3) and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) (4) were conducted on the fermented samples. Hemicellulose content was estimated as the difference between NDF and ADF while cellulose content was estimated as the difference between ADF and ADL.

Table 1. Proximate composition (% DM) of solid state fermented RS with different supplementation and filamentous fungi.

Treatment	Fungi	ADF	NDF	ADL	Dry matter	Crude protein	Ash	Cellulose	Hemi-cellulose
RS	*A. niger*	53.7^{jkl}	76.3^{l}	12.8^{a}	97.2^{a}	7.3^{ghi}	12.4^{ij}	40.9	22.6
	T. harzianum	54.6^{kl}	76.7^{l}	12.6^{ab}	96.6^{b}	7.0^{ijk}	12.3^{jkl}	42.1	22.1
	R. oligosporus	54.6^{kl}	76.2^{l}	12.0^{cde}	96.1^{hi}	6.3^{kl}	12.2^{klm}	42.6	21.6
	Control	55.1^{l}	82.5^{n}	11.5^{fgh}	95.1^{p}	5.9^{l}	12.1^{m}	43.6	27.4
RS + Urea	*A. niger*	52.9^{ijh}	68.5^{de}	12.0^{cde}	97.1^{a}	9.0^{d}	12.9^{fde}	40.9	15.6
	T. harzianum	53.8^{jkl}	69.8^{fg}	11.8^{def}	96.8^{b}	8.3^{def}	12.7^{gf}	42.0	16.0
	R. oligosporus	53.6^{jkl}	72.8	11.8^{def}	95.9^{ij}	8.0^{efg}	12.3^{ijk}	41.7	19.3
	Control	54.6^{kl}	78.7^{m}	11.0^{k}	95.5^{nm}	6.9^{jkl}	12.2^{klm}	43.6	24.1
RS+ Molasses	*A. niger*	53.7^{jkl}	70.3^{g}	12.5^{ab}	96.3^{ef}	7.9^{fgh}	12.4^{ih}	41.2	16.6
	T. harzianum	53.7^{jkl}	73.1^{j}	12.6^{ccl}	95.9^{j}	7.7^{ghi}	12.4^{ij}	41.6	19.4
	R. oligosporus	53.4^{ijkl}	73.2^{j}	11.8^{efg}	95.7^{k}	7.6^{ghi}	12.2^{klm}	41.7	19.8
	Control	54.5^{kl}	81.9^{n}	11.4^{hij}	95.3^{o}	6.7^{jk}	12.1^{m}	43.1	17.4
RS+ PKC	*A. niger*	46.2^{a}	64.9^{a}	12.4^{ab}	96.8^{b}	12.2^{a}	13.8^{a}	33.8	18.7
	T. harzianum	48.6^{bc}	65.3^{ab}	12.3^{bc}	95.4^{n}	11.7^{ab}	13.0^{ccl}	36.3	16.7
	R. oligosporus	48.8^{bcd}	68.1^{d}	12.0^{cde}	95.1^{p}	11.6^{ab}	12.1^{cde}	36.8	19.4
	Control	51.1^{fgh}	71.6^{hi}	11.5^{fgh}	94.6^{q}	8.1^{efg}	13.0^{ml}	39.6	20.4
SE diff		0.324*	0.410*	0.721*	0.476*	0.623*	0.551*	0.031*	0.042*

Means with different superscripts in the same column are significantly different at $p<0.05$.

*: significantly different at p<0.01.

Fermentation had significant (P<0.01) effect on ADF, NDF, ADL, cellulose, hemicelluloses, CP, DM and ash. Among the fungi, *A. niger* showed the highest reduction in ADF (from 55.1 to 46.2%) and NDF (from 82.5 to 64.9%) when supplemented with PKC. This was due to the reduction of cellulose and hemicelluloses contents of RS after fermentation (Table 1) and clear indication of secretion of cellulose/hemicelluloses-degrading enzymes by the fungus (5). The crude protein (CP) content increased from 5.9 to 11.6, 11.7 and 12.2% by *R. oligosporus, T. harzianum* and *A. niger*, respectively. The increase in CP may be partially attributed of fungal protein, specifically chitin (6). The ash contents and DM of fermented samples increased at varying degrees. The present results suggest that *A. niger* appeared to be the best of the three fungi in improving the crude protein content and fiber reduction. It can be concluded that *A. niger* and *R. oligosporus*; with PKC supplementation will be useful in value-addition of rice straw for feeding ruminants.

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EFFECT OF OXYGEN ON CLA PRODUCTION BY WASHED CELLS OF LACTIC ACID BACTERIA FROM RUMEN AND POULTRY

S.T. Yong[1], S.W. Zuhainis[1*], C.C. Sieo[1,2] and Y.W. Ho[2]

[1]Faculty of Biotechnology and Biomolecular Sciences. [2]Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor.

*Email: zuhainis@biotech.upm.edu.my

Conjugated linoleic acid (CLA) is a fatty acid that contains 18-carbon with a double conjugated bond. It has been proven in several cell culture systems and animal models to have the potential in anti-cancer, anti-inflammatory, anti-atherosclerosis, anti-obesity and modulation of immune system (2). Consumption of (CLA) which produced by lactic acid bacteria (LAB) was believed to be one of the ways to improve the quality of health (1). Naturally, CLA can be found in large quantities in ruminant meat, milk, cheese and to a lesser degree in egg yolk and chicken (3). CLA can be produced as intermediate of biohydrogenation pathway in the rumen of ruminant by certain microorganisms under anaerobic condition and CLA production by *Lactobacillus acidophilus* was low in the presence of oxygen (4). However, some studies showed that *Butyrivibrio fibrisolvens* and *L. reuteri* exhibited a higher CLA formation in aerobic than anaerobic condition (5,6). Therefore, the objective of this study is to examine the effect of oxygen on the production of CLA by the washed cell of LAB.

In the experiment, CLA-producing LAB was isolated from gastrointestinal of chickens and rumen fluid of cattle. Reactions were carried out under aerobic and anaerobic conditions. Cultures of 24 h of *L. salivarius*, *Enterococcus faecium*, *L. agilis* and *Streptococcus bovis* were tested. All the treatments were incubated in a pH 6 buffer at 37°C for 0, 6, 12, 24, 36, 48, 60 and 72 h, respectively. A 0.5 mg/ml of linoleic acid (LA) was used as the substrate and 1% wet cell (w/v) was washed under both anaerobic and aerobic conditions. Lipid isolation from the culture media was then carried out and the total CLA produced were quantified at a wavelength of 233 nm in a UV spectrophotometer (7).

Figures 1 and 2 shows that all the four LAB strains examined have better CLA concentration under aerobic conditions than anaerobic conditions throughout the 72 h reaction period. Quantity of CLA increased markedly and reached maximum level at 48 h and then decreased continuously in further reaction time. This is may be due to the increased of CLA distributed extracellularly as the reaction time proceeded. Based on the biohydrogenation pathway, LA were believed to be converted to hydrogenated products by the four strains of LAB under anaerobic condition, hence, a relatively low concentration of CLA was detected. In conclusion, *L. salivarius*, *Enterococcus faecium*, *L. agilis* and *Streptococcus bovis* could produce CLA from LA under aerobic and anaerobic conditions and CLA formation is more effective under aerobic condition.

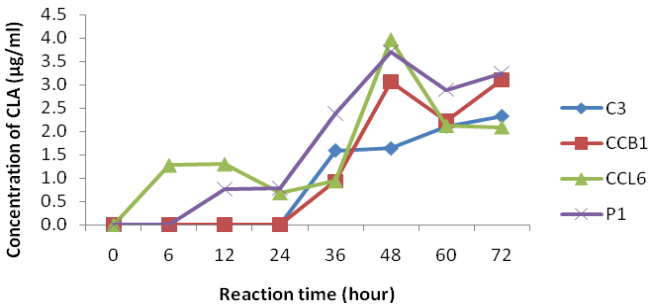


Figure 1. Production of CLA by washed cell of LAB under anaerobic condition. C3= *Streptococcus bovis*, CCB1= *L. salivarius*, CCL6= *L. agilis and* P1= *Enterococcus faecium*.

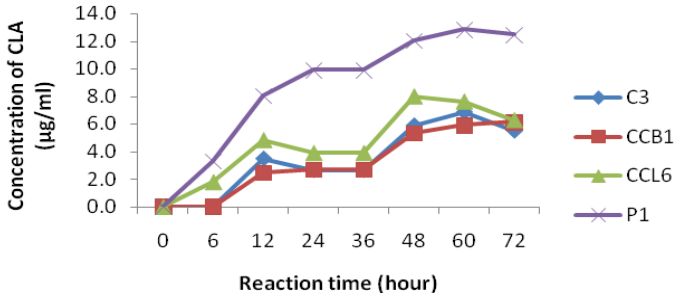


Figure 2. Production of CLA by washed cell of LAB under aerobic condition. C3= *Streptococcus bovis*, CCB1= *L. salivarius*, CCL6= *L. agilis and* P1= *Enterococcus faecium*.

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IMPROVING THE NUTRITIVE VALUE OF PALM KERNEL CAKE (PKC) THROUGH CHEMICAL PRE-TREATMENT AND FUNGAL FERMENTATION

M.M. A'dilah and A.R. Alimon*
Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400 Selangor
*Email:ralimon@agri.upm.edu.my

An experimentwas conducted to determine the effect of chemical pre-treatment and solid state fermentation (SSF) using *Rhizopus oligosporus*or or *Trichoderma harzianum* on the nutritive value of Palm Kernel Cake (PKC). PKC is an important by-product of the palm oil industry and is an excellent source of energy and protein for ruminants. However, PKC is high in fibre and moderate in protein content and not suitable for poultry (1). Livestock feed from palm products presents an excellent opportunity for replacing imported feed like corn and soybean meal. In this study, PKC was pre-treated with alkali and acid, followed by SSF with the objective of reducing its fibre content and increasing protein content (2). PKC was treated with either ammonium hydroxide or a combination of formic acid and acetic acid. PKC was soaked in the respective solutions for 2 to 24 hours and dried in a forced draught oven. After the pre-treatments the PKC was fermented with either *Rhizopus oligosporus* or *Trichoderma harzianum*. Chemical pre treatment improved the nutritive value of PKC in that it reduced the NDF and ADL content of PKC. NDF content reduce from 76.2% to 68% when soaked with ammonium hydroxide. ADL content reduce from 6.8% to 4.7% when soaked with formic acid and acetic acid and reduced until 3.8% when soaked with ammonium hydroxide (Table 1). On the other hand, pre-treatments also significantly ($p<0.05$) reduced the crude protein content. This was probably due to the dissolution of protein by the chemicals and also due the increase in the percentage of ADF. Following the chemical pre-treatments, SSF of PKC further increased the crude protein content but the increase was not significant as compared to the untreated PKC.

Table 1. The effect of chemical pre-treatments (FA+AC; ammonium hydroxide) on the proximate composition (%) of PKC.

Nutrients (% DM)	Untreated PKC	PKC (FA+AC)	PKC (NH4OH)	Level of Sig
DM	89.6±0.06	90.1± 2.3	86.3±0.01	NS
Ash	4.2±0.09	4.1±0.05	4.0±0.01	NS
CP	15.0±0.30^{a}	13.2±0.68^{b}	12.5±0.62^{b}	*
NDF	76.2±3.16^{ab}	86.5±3.62^{a}	68.0±7.19^{b}	*
ADF	53.7±7.68^{b}	79.1±2.27^{a}	69.26±7.33^{ab}	*
ADL	6.8±1.82	4.7±3.41	3.8±3.67	NS

NS, not significant; * significantly different at $p<0.05$. ^{a,b} Means with different superscripts are significantly different

This suggests that pre-treatment may not be recommended due to the reduction of crude protein. SSF after pre-treatment only increased the crude protein to a level similar to the initial concentration in the untreated PKC. Nevertheless, the SSF with the

two fungus reduced further the NDF, ADF and ADL content of PKC (Table 2). NDF content reduced from 73.2% to 64.9% while ADF content from 55% to 45.7% and ADL content from 3.9% to 1.2%. With regards to the protein content, pre-treatment with ammonium hydroxide or with acids did not change the CP content of PKC. Similarly, fermentation with either *T. harzianum*or or *R. oligosporus* did not affect CP. It was possible that the duration of 7 days fermentation may be not sufficient time for the optimal growth of the fungi to elucidate any effects. In conclusion, the combination methods of chemical pre-treatment and SSF appeared to improve the nutritive value of PKC through reduction in the fibre fractions but not the protein fraction. Further work is needed to examine the effect of these treatments on the feeding value and palatability of treated PKC for poultry.

Table 2. The effect of SSF with *Trichoderma harzianum* or *Rhizopus oligosporus* on the proximate composition (%) of untreated and chemically pre-treated PKC

Nutrients	Main Effect of Pre-treatment			Sig	Main Effect of Fungus		Sig
	Untreated	FA+AC	NH40H		Rhizopus	Trichoderma	
DM	95.9	95.8	95.5		95.5	9.9	
Ash	6.6±0.32	6.4±0.31	7.0±0.36	NS	6.6±0.32	6.8±0.23	NS
CP	16.9±0.29	16.1±0.31	16.7±0.26	NS	16.4±0.26	16.7±0.22	NS
NDF	72.3±1.0	70.6±1.6	67.8±2.7	NS	72.5±1.1a	68.9±1.5	*
ADF	51.3±1.1	50.6±1.9	51.1±3.3	NS	48.8±1.7	53.1±1.3	NS
ADL	2.4±0.29	2.6±0.43	2.3±2.1	NS	2.4±0.33	2.5±0.32	NS

NS, not significantly different; * significantly different ($p<0.05$)

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BODY WEIGHT AND BODY CONFORMATION ASPECTS OF CYPRUS SHAMI GOATS IN MALAYSIA

M. Murugaiyah*, M.H. Hamdan and F.F.A. Jesse
Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor.
*Email: muru@vet.upm.edu.my

Cyprus Shami goat is known worldwide as Damascus goat, is a mixed meat and dairy goat breed known for its high milk yield, meat and reproductive performance in Near and Middle East countries such as Turkey, Syria, Lebanon, Jordan, Israel, Cyprus, etc. (1). This goat can be characterised by reddish brown coat colour consisting mostly long hair. This breed was introduced to Malaysia at the end of 2009. The objective for importation by Malaysian government is because of its dual purpose for its meat and milk qualities. There have been few studies carried out on linear body measurements on Cyprus Shami goats for their possible use for estimating goats' live weights (2, 3). This paper reports a study on the linear body measurement to predict body weights in different age groups of Cyprus Shami goats.

The study involved a total of 30 Shami goats from Shami Goat Sdn. Bhd, Chembong, Negri Sembilan. The goats were divided into three groups: group 1 (6 to 12 months old), group B (12 to 18 months old) and group C (18 months old and above). Age of the animals was obtained from their birth date records or estimated based on the number of permanent incisors present. Live weight of goats was recorded using a weighing scale. The height at withers was measured from the bottom of the front foot to the highest point of withers between the shoulders by using a girth tape. The body length was measured as the distance from the point of shoulder to the posterior edge of the pin bone (tuber ischii) using a girth tape. The heart girth was measured as the circumference of the body immediately behind the shoulder blades in a vertical plane, perpendicular to the long axis of the body using a girth tape (4).

Data was analysed using SPSS version 16.0 via two ways ANOVA. Separate models were developed to determine the combination of body conformation parameter (height at withers, body length and heart girth) that explains variation in the dependent variables (body weight).

Table 1. The predictive equation and coefficient of determination (R^2) for body weights using height at withers (HW), body length (BL) and heart girth (HG) in Cyprus Shami goats.

Variables	Regression equations	R^2
HW	Y = -165.06 + 2.819 HW	0.677
BL	Y = -74.52 + 2.081 BL	0.802
HG	Y = -50.388 + 1.158 HG	0.482
HW+BL	Y = -132.702 + 1.239 HW + 1.467 BL	0.862
HW+HG	Y = -162.154 + 2.173 HW + 0.527 HG	0.741
BL+HG	Y= -73.036 + 2.157 BL + -0.069 HG	0.802
HW+BL+HG	Y = -130.955 + 1.246 HW + 1.57 BL + -0.097 HG	0.864

The mean value for each body confirmation parameter in the Cyprus Shami goats showed a higher mean value and a highly significant difference ($p<0.05$)

compared to what had been reported by (3). This significance may be due to associated factors such as: nutrition, management system and environmental stress.

In predicting body weights using linear body measurements, the study showed that the best combination is the three parameters namely; height at withers, body length and heart girth. This is because of the highest R^2 value was obtained (0.864) using this multiple combinations. This differs from that reported on Red Sakoto goats by (5), where only heart girth and body length were used individually in estimating the body weight of goats. The present predictive equation has limitations because for one the study was done only on males and no measurements were done on females and pregnant animals. Further, since nutrition is a major factor in the growth of the animals, there is a possibility that the diet in this farm (a mixture of oil palm frond, forest leaves, brew and soya hull) could have had an impact on the growth and may not apply to goats fed on a different diet from the one stated here. In general, majority of goat famers in Malaysia feed chopped Napier grass and palm kernel concentrates to their goats.

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ENTRY POINT PROJECTS (EPPS) IN LIVE STOCK SUBSECTOR

S. Noor Rahim*
Livestock Commodity Development Division, Department of Veterinary Services, Putrajaya
*E-mail: norahim@dvs.gov.my

Economic Transformation Program (ETP), which has been launch on October 2010, is a comprehensive effort that will transform Malaysia into high-income nation by 2020. This program provides strong focus on 12 National key Economic Areas (NKEAs) to make substantial contribution to Malaysia's economic performance. Agriculture sectors is one of the NKEAs that will be transformed into agribusiness through 16 Entry Point Projects (EPPs) that catalyze the establishment of market driven, industrial scale and integrated agriculture. Five EPPs are related to livestock farming and DVS is given task as the owner of these EPPs. EPPs in livestock are expending the production of swiftlet nests, (EPP2), Rearing cattle in oil palm estate (EPP5), Strengthening current anchor Companies in Feedlot Operation(EPP12), Partnering with foreign company in dairy Cluster (EPP 13) and Investing in Foreign Cattle Farming (EPP 16).

By the year 2020 EPPs in livestock subsector will contribute RM 5.276 billion to GNI and created 27,200 job opportunities. Total investment is RM 4.786 billion which will be contributed mainly by private sectors. Public funding is only RM 512 million or 10.7% of total investment. Contribution and investment for each EPP is as Table 1.

EPP2 will focus in increasing productivity and adding new premises across the country to capture 30% of the total market worldwide, and increasing downstream products to increase revenue. EPP 5 will create viable model in cattle integration within 2 years and the success model will be replicated to other plantation. EPP 12 will create more anchor companies to achieve targeted self-sufficiency level with complete value chain in beef production. EPP 13 will focus on developing large scale dairy farm with down stream activities to increase profit due to low margin of dairy cattle farming. EPP 16 will focus in encouraging local player to invest in oversea in livestock operation for reverse investment as well as for global market.

Table 1 : Contribution and investment of EPP in livestock subsector

EPP No.	Total Investment (RM Million)	Public Funding (RM Million)	Private Sector Investment (RM Million)	GNI (RM Million)	Job Created
EPP2	1800	64	1,736	4,500	20,800
EPP5	343	143	200	150	3600
EPP12	634	121	513	183	2000
EPP13	709	184	525	326	800
EPP16	1300	0	1300	117	0
Total	4,786	512	4,274	5,276	27,200

The role of public sector through DVS is to facilitate and to provide conducive business environment for private sector to excel in their business. Project detail activities of DVS in each EPPs are as Table 2.

Table 2 : EPP's Project Detail

EPP No.	Detail Activities
EPP2	Increase production of upstream by building of additional 2,000 new premises annually and 6 Collection Centers, improve monitoring and enforcement of Good Animal Husbandry Practice (GAHP), and Good Manufacturing Practice (GMP) for swiftlet premises and processing plants by incorporating traceability system and increase downstream product development and cleaning of more raw products locally by construction of R&D Centre for development and processing
EPP5	Integration of another 300,000 heads of cattle in large oil palm plantations at 3-4 hectares per head, emphasize on systematic rotational grazing and adherence to GAHP and breeding program for the cattle while synergizing with the estate owners.
EPP12	Establishment of 300 satellite farms to support the current anchor company, National Feedlot Corporation and other potential anchors for feedlot operations, Anchor company will oversee the operations of the entire value chain while focusing on the integrated and consolidated downstream processing and downstream operations will be improved by enforcing certification of abattoir, cold-chain delivery of chilled cuts, promoting market awareness of buffalo meat and beef, and developing beef byproducts.
EPP13	Establishment of dairy clusters led by anchor companies to produce milk on a large-scale basis and oversee the operations from production to marketing and upgrading of the existing cold-chain milk facilities to ensure the quality of milk from farm to market is maintained
EPP16	Secure a consistent and affordable source of live animals for breeding, feedlot, and dairy operations over the next 10 years. The scope has been expanded to include other potential areas in livestock e.g poultry with purpose of bringing in profit into the country

In conclusion, successfully implemented EPPs will transform livestock subsector into agribusiness, moving toward economies of scale, value chain integration, premium down stream product and enable to capture global market.

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THE REGIONAL COMPETITIVENESS OF MEAT AND MEAT PREPARATION SUB-SECTORS

W.M.M. Wan Bakar*, M.M. Ismail and A.M. Abdullah
Department of Agribusiness and Information System, UPM Serdang, Selangor.
*Email: wmarzuki@putra.upm.edu.my

Over the years, Malaysia has recorded a substantial and persistent food trade deficit. It continues to grow at an alarming rate as the deficits climbed to a staggering RM 10.1 billion in 2008 (1). Registered at RM3.4 billion in 2005, it swelled to RM 4.9 billion in 2000. If this detrimental trend continues unimpeded, the food trade deficit is expected to reach RM12.4 billion by 2010 with food imports at RM27.3 billion, far outweighing exports worth RM14.9 billion. In line with the global oil and food crisis that emerged somewhere in July 2007, it has heightened the government's awareness and brought this issue to the forefront of public attention. This phenomenon has to be arrested which requires Malaysia to boost its food exports. It is the target of the Ministry of Agriculture and Agro-based Industry (MoA) to achieve a food trade surplus of RM 1.2 billion by 2010 with exports amounting RM 15.5 billion which would exceed the RM 14.3 billion value of imports (2).

To achieve the above-mentioned target, there is a need to determine the local commodities that are capable of substituting imported products while at the same time, a requirement to search for new export products. For that, it is important for Malaysian food processors to identify food sectors that are internationally competitive.

International competitiveness is partly influenced by the macro-economic environment, the ability to reduce costs using new technology, improved product quality and the development of new products and the ability to successfully increase market shares (3). Competitiveness is a complex process which derives from strategies adopted at the firm level, industry level, sector level and wider macro-policies implemented at the national level. Apart from that, economic integration or liberalization also has a major impact on foreign trade and the allocation of economic activities. Differences in comparative advantage across countries determine specialization patterns at the inter-country level and it works even in the absence of factor mobility across nations. A nation is said to have a comparative advantage in producing a particular product if it can produce at lower relative costs than others, and in trade analysis, competitiveness is reflected in products that reveal a high comparative advantage.

In this study, the meat and meat preparation sector has been chosen. While it is mainly an imported item it can be re-exported into higher value added products and thus it is possible to convert the existing threat (high food deficits) into an opportunity (potential exports). In this paper, we look forward to ascertain whether Malaysia, as well as other selected ASEAN countries, is an efficient producer (especially in adding value) of meat and meat preparation in terms of revealed comparative advantage and competitiveness. It is the aim of this study to evaluate the competitiveness of 17 food commodities in the meat and meat preparation sector (division 01) covering the period of 1998 to 2007. The data cover commodities in the HS 6 digits group which include the sub-group of bovine, sheep, lamb and goat.

The findings of the study indicate that Singapore has a competitive advantage in 11 commodities while Malaysia has a competitive advantage in 4 commodities, which

are bovine carcasses and half carcasses, fresh or chilled, bovine cuts boneless, fresh or chilled, bovine cuts boneless, frozen and bovine meat cured. Singapore has competitive advantage in those commodities mainly due to its efficient value adding and re-exporting activities. However, it appears that Malaysia has a competitive disadvantage in all of the other commodities in the 01 division. The absolute comparative advantage possessed by Singapore in the ruminant sector of meat and meat preparation is quite worrying for Malaysia. By focusing on *halal* meat, Malaysia is expected to gain considerable comparative advantage, but the figures showed otherwise. If Malaysia cannot increase its market share in the ruminant category, then all of the money spent in making Malaysia a leading *halal* hub of the world would have been ineffective.

A time trend regression function was fitted to each commodity throughout the sample period. The commodities were divided into two categories which are bovine, and sheep, lamb and goat. For the bovine category, Indonesia and Malaysia exhibit 2 positive trend lines while the rest are negative. The Philippines exhibits 2 positive and 1 negative trend lines, while 7 positive and 1 negative trend lines were displayed by Singapore, and 4 positive and 5 negative trend lines were exhibited by Thailand. For the sheep, lamb and goat categories, Indonesia shows 3 positive and 4 negative trend lines, whereas for Malaysia the trend lines appeared to be 3 positive and 4 negative. Singapore maintained its position by having the highest number of significant positive trend lines with 5 commodities followed by Thailand with 4 positives and 2 negatives. This again reemphasizes our position in *halal* meat trade. Malaysia and Indonesia seem to be losing their competitive advantage while Singapore and Thailand seem to be gaining a competitive advantage. The trend lines analysis also indicates changing competitiveness where the new player in the meat and meat preparation sector is Thailand, and Singapore is still leading the pack.

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THE EFFECT OF FEEDING SUPPLEMENTARY MONENSIN ON COCCIDIOSIS INFECTION AND AVERAGE DAILY GAIN (ADG) IN GOATS REARED UNDER INTENSIVE SYSTEM

Z. Noor Izzah [1*], K. D. S. Kanwal [1] and A. Punimin [2]

[1]School of Sustainable Agriculture, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia; [2]Department of Veterinary Service and Animal Industry, Level 3, Block B, Wisma Pertanian Sabah, Jalan Tasik Luyang, Locked Bag 2051, 88999 Kota Kinabalu, Sabah, Malaysia
*Email: nizzah@ums.edu.my

Goat is considered as a multipurpose animal; producing meat, milk, skin and hair. In Sabah, goat is used mainly for meat production (1). In 2008, the self-sufficiency level, per capita consumption and industry growth rate in Malaysian goat industry were 9.15%, 0.76 kg, and 9.7%, respectively (2). The goat population in Sabah was estimated at about 30,000 heads in 2006 (3). Smallholders owned about 80% of the goat population in Sabah, and most of them maintain their animals on native pastures grown on the roadside in the villages (1). The main problems faced by the goat farmers were lack of base breeder population of goats, lack of nutritious forages, and poor health and feeding management practices. These problems would subsequently hinder the development of goat production in Sabah.

Another serious problem is the infestation with external and internal parasites leading to poor growth rate in the animals. One of the problems faced by the industry is the high percentage of coccidiosis infection (4). Coccidiosis is an internal parasite seriously affecting the economic and productivity of the farm due to its effect on the growth and weight gain in goats reared specially under intensive system. Once an animal is infected with coccidiosis, there will be permanent effects on its growth rate resulting in stunted, ill-faring kids and even lead to death (5). These factors would result into low animal productivity and lower farm economic returns. Researchers in animal industry are interested to find an effective method that can eliminate the parasites and also promotes the animal growth performance. Sodium monensin ionophore, which is produced by fermentation of *Streptomyces cinnamonensis* bacteria (6), is getting much attention in the livestock industry as an anticoccidia drug that promotes better animal growth performance.

In this study, monensin supplementation at the rate of 200mg/kg of feed was fed for 5 months to 200 males and females of Kalahari Red breed, which were divided into various groups according to the age (nursing kid, weaning kid, yearling goat and breeder goat). There was no monensin toxicity observed in the animals. All animals showed increase in body live weight after supplementation with monensin. Male yearlings had insignificant increase in ADG (0.10kg/day). Both female yearling (0.10kg/day, $t=-3.63$, $p<0.05$) and female breeder goats (0.01kg/day, $t=-2.16$, $p<0.05$) showed significant increase in ADG following supplementation with monensin. It was concluded that monensin is highly efficient in increasing ADG in goats with low daily gain (7). The study showed that the most affected group was that of yearling goats where the coccidiosis infection was under control and the ADG was the highest. Therefore, it may be suggested to use strategic monensin supplementation in yearling goats for them to gain desirable market weight in shortest possible time.

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SURVEY OF COPPER TOXICITY IN DORPER BREED SHEEP: REVIEW ON MANAGEMENT AND DISEASE APPROACH IN SHEEP

S. Gunalan*, A. Shah, M. Hishamfariz, M. Suhaimi, A.R. Rozita, K. Rosilawati, Wan S. Ramli

Regional Veterinary Diagnostic Laboratory Kuantan, Jalan Sri Kemunting 2, Kuantan, Pahang
*Email: gunalan@dvs.gov.my

Although the Malaysian livestock industry, particularly pertaining to sheep has increased tremendously and research towards improving productivity has been focused on the production systems and nutrition. This includes intensive farming system with silage feeding, cut and carry system and the use of agricultural by-products especially palm kernel cake (PKC) as sources of feed. Investigation into trace elements nutrition or nutritional pathology has however been insignificant and this is the most neglected field of research in sheep. This is reflected by an increase in unsolved cases of ill-thrift, infertility, abortion and pica nationwide. It is believed that trace mineral imbalances are major constraints contributing to the problems. In this study, survey on the copper toxicity in Dorper breed sheep has been carried out to determine the factors that contribute and possible clinical sign and post mortem lesion that may present.

Toxicological results must be interpreted in relation to history, clinical findings and histopathology. The liver is the central organ in copper metabolism, by removing ionic copper from blood, disposing a portion into bile, synthesizing ceruloplasmin and storing the remainder. Thus, liver will be a good organ indicator for tissue copper residue in animals. Kidney is considered superior to liver for biochemistry in acute and chronic copper poisoning. Kidney copper concentrations rise faster in acute toxicosis. According to most of literature, more data available for liver / hepatic copper concentration as tissue copper residue that has been adapted in this study.

Most of kidney showed gunmetal appearances and severe necrosis in liver specimen. Few sheep have showed sign of anemia in the blood haemogram and increased in biochemistry parameters (liver enzymes) in the flock that may succumb with copper toxicity. Thus in this survey the tissue copper residue from the post mortem sample has been taken to support the evidence of copper toxicity in the Dorper breed sheep.

In this study, 13 cases of mortality in Dorper sheep involved the feeding of grower pellet with reported level of copper in feed range 9.15 to 25.21 ppm. The clinical sign were reported as generalized weakness, inappetance, pale mucous membrane, shock, dehydration, ill-thrift condition, limb shivering, muscular spasm and lameness due to hind limb weakness. The morbidity and mortality rate almost 10-15% in the farm. The post mortem lesions were icterus carcass, hepatomegaly and friable liver, gunmetal-colored kidney, enlarged spleen and severe congestion of organs. The histopathologies of the liver samples were showing lesion such as severe hepatic necrosis, renal tubular necrosis and severe congestion were taken place in various tissues (1). The liver samples contained copper level ranges between 69.62 to 500 ppm (as 6 samples showed more than 500 ppm) with an average of 344.32 ppm. In the kidney the copper level also ranges between 42.21 to 362.68 ppm with an average of 131.79 ppm. Based on the results, it can be suggested that the Dorper sheep has the less tolerance to high copper level. If the feed contain 10-20 ppm copper and the molybdenum level is low (less than 1 ppm), the hepatic copper accumulation in sheep liver will occur. The chronic copper episode will cause inability of sheep to increase the biliary excretion of copper in response to dietary increase of feed

contain high level of copper. The mechanism involved was high concentration of copper cause lipid peroxidation of mitochondrial membranes leading to severe hepatic necrosis and copper released into bloodstream. This may lead to hemolysis of red blood cells (RBCs) by ionic copper and this hemolytic toxic effect will take place that may damage other organs including direct effect to kidney (2). As described in Merck Veterinary Manual, the blood and liver copper concentrations are increased during the hemolytic period with liver concentrations more than 150 ppm (wet weight) will be significant finding in sheep. Thus, the concentration of copper in the tissue for the Dorper sheep in this farm will correspond well with the copper level and sign of copper toxicity. In one of the chronic copper toxicity in adult Dorper sheep, there was serous atrophy in the heart muscles, hepatomegaly, and severe congestion of vital organs during post mortem. The blood picture showed 3.77 ug/ml copper levels in whole blood (EDTA tube). And the sheep have showed sign of lymphocytosis (in hematology parameter) and hyperglobinemia (in biochemistry analysis). The liver enzymes (such as Aspartate aminotransferase, Total bilirubin, Gamma-glutamyl transferase and Lactate dehydrogenase) have increased significantly.

Separate studies should be done in this particular breed sheep before such assumption that the copper toxicity has been major contributing factor for the morbidity and mortality of the sheep in this farm. The accuracy of this study questionable as the necropsied liver sample instead of biopsy or plasma sample that may vary in copper level as the post mortem effect take place according to time of examination and frozen effect of organ samples. And, the infectious agents can modify the overall status of trace elements in sheep (3) as we lacking information in this matter. Thus, in monting an investigative program in trace element research, the role of infectious and non infectious agents should be considered. The diagnosis of copper status in flock of sheep should be based on the combination of analysis and interpretation of herd history, clinical signs, and individual necropsy findings, laboratory tests on serum (ceruloplasmin level) and fresh liver samples whenever possible. Further study can be done with priority given to determine copper in liver based on breed, age, sex, dietary regime and purpose using larger sample size.

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EFFECTS OF SEAWEED EXTRACTS ON LIPID PROFILE OF HIGH-SUGAR, HIGH-FAT AND STREPTOZOTOCIN-INDUCED TYPE TWO DIABETIC RATS

M. Moteshakeri[1], M. Ebrahimi[2] and S. Mohamed[1*]
[1]Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangore. [2]Faculty of Veterinary Medicine, Universiti Putra Malaysia, Selangor.
[*]Email: mohamed.suhaila@gmail.com

Diabetes mellitus is a public health problem in Malaysia that is increasing, serious and costly. It is suffered by more than 180 million people worldwide while this number is expected to increase at least 366 million by year 2030 (1). Type two diabetes is characterized by abnormalities in carbohydrate, fat and protein metabolism, which lead to hyperglyceamia and hyperlipidemia. The main reasons of morbidity and mortality in the diabetic patients are heart disease and stroke as patients are two to four times more susceptible to have heart disease and 5 times more likely to have stroke (2, 3). Thus, it is important to prevent or delay complications with adequate pharmacological (chemical or herbal drugs) and non-pharmacological (weight loss or diet management programs and daily exercise) treatments.

Since synthetic medications have limited efficiency with various adverse effects, there have been increasing interests in the use of medicinal plants. Recently, seaweeds have been the center of focus as a natural source of biological compounds useful for human health (4). Brown seaweed, *Sargassum polycystum* (SP) has been reported to be rich in biologically active substances with potential healing effects. High calorie diets following low-dose streptozotocin has been found to mimic natural history (transition from insulin resistance to β-cell dysfunction) of the disease induced in Sprague-Dawley rats. We investigated hypolipidemic effects of ethanolic and water extracts of SP in high-sugar, high-fat diet-fed and low-dose streptozotocin (STZ)-induced type two diabetic rat model.

Male Sprague–Dawley rats (200–250 g) were divided into seven groups (n=8): normal control group (NC) fed standard rodent diet, diabetic control group (DC), diabetic rats treated with ethanol extract 300 mg/kg (DE300), diabetic rats treated with ethanol extract 150 mg/kg (DE150), diabetic rats treated with water extract 300 mg/kg (DW300), diabetic rats treated with water extract 150 mg/kg (DW150), diabetic rats treated with metformin (DM). Diabetes was induced in all diabetic rats by feeding high-sugar, high-fat diet (4.6 kcal/g) known as western diet for 16 weeks and then intraperitoneal injection of 35 mg/kg body weight of streptozotocin.

After 22-days seaweed treatment, all seaweed extracts reduced total cholesterol (TC) and triglyceride (TG) level significantly ($P<0.05$) compared to diabetic control (DC) group. Low density lipoprotein-cholesterol (LDL-C) level was significantly ($P<0.05$) reduced in DW300 group compared to DC group. High density lipoprotein-cholesterol (HDL-C) did not change significantly ($P>0.05$) in all treated groups.

HDL-C/TC ratio improved in all treated groups but in DE150, DW300, DW150 and DM groups the improvement was statistically significant ($P<0.05$) compared to DC group. Therefore, seaweed may be useful to diabetic patients with atherosclerosis, since the seaweed has been known for its therapeutically bioactive compounds such as phlorotanins (carotenoids and teriterpenoids), alginate and fucoidan.

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TOXICOLOGICAL EVALUATION OF *JATROPHA CURCAS* OIL SEED CAKE IN SPRAGUE DAWLEY RATS: HAEMATOLOGICAL PROFILES

M.S. Siti Masidayu, S.M. Rosly, Y. Nor Idayusni, K. Subramaniam, A. Sivapragasm[1] and S. Shanmugavelu*

Strategic Livestock Research Centre, Malaysia Agricultural Research and Development Institute, GPO Box 12301, 50774 Kuala Lumpur, Malaysia, [1]CABI Southeast and East Asia, P.O. Box 210, Serdang, Selangor, Malaysia;

*Email: shan@mardi.gov.my

Jatropha species belong to the family Euphorbiaceae and have been used as traditional medicine for various ailments such as fever, mouth infections, jaundice, worm sores and joint rheumatism. The search for alternate fuel in the form of biodiesel resulted in large-scale planting of Jatropha in India, China, Madagascar, Myanmar and many other developing countries (1) The oil is generally produced from whole seeds using a screw press resulted a seed cake by-product which is high in crude protein (2) and a potential feed for animals. However, *Jatropha* species contain numerous phytochemicals such alkaloids, saponins and tannins with potent antimicrobial effects as well as phorbol esters which are toxic to a number of animal species (3). Nevertheless, there have been some varieties of *Jatropha* that are less toxic to animals (4). The aim of the present study was to evaluate the toxicological effects of Sprague Dawley rats fed with locally grown and processed *Jatropha curcas* oil seed cake.

A total of 30 male Sprague Dawley rats with an average body weight of 230g were subjected to 5 treatment groups in a completely randomized block design (RCBD). The treatment groups were fed *Jatropha curcas* oil seed cake in the feed at 0, 100, 500, 2000 and 5000 mg/kg body weight for four weeks. The rats were acclimatized to the housing conditions for 2 weeks prior to treatment diets (temperature between 25-27°C; relative humidity at 40-70% and 12-h light/dark cycle regime). Rats were kept individually in a polycarbonate mesh bottom cage. The rats were observed daily for clinical signs and mortality and subjected to a full necropsy after four weeks. Blood samples were collected for haematology, clinical chemistry and histopathology analysis. Data were analysed using SAS version 9.10, and only haematological parameters are reported in this paper.

At the end of the study, all rats were observed to be healthy with no gross pathological lesions. Similarly there were no significant changes in body weight gain or feed intake (data not shown). In general, the blood haematology was within the normal range for all groups. However, there were significant changes in the red blood cell (RBC) parameters (Table 1) in rats fed with *Jatropha curcas* oil seed cake. There was a significant reduction in the total RBC, HB (haemoglobin) and PCV (packed cell volume) when *Jatropha curcas* oil seed cake was included at 5000 mg/kg body weight. In fact there was a linear deterioration of RBC parameters with increasing concentration of *Jatropha curcas* oil seed cake in the diet which is suggestive of toxic effects anaemia as observed by other studies using normal (5) or detoxified *Jatropha* (6). In the present study, the toxic effects were only apparent at the higher dosage of 5000mg/kg body weight which is equivalent to 0.5% body weight or 5% in the diet. It is apparent that if *Jatropha curcas* oil seed cake is to be utilised for its high protein content in animal diets it has to be detoxified (7).

Table 1: Haematological profile of Sprague Dawley rats fed *Jatropha curcas* oil seed cake

Parameters	*Jatropha curcas* oil seed cake (per kg body weight)										P value
	0 mg/kg		100 mg		500 mg		2000 mg		5000 mg		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
WBC (10^3/µl)	9.2	0.97	11.2	0.84	9.7	1.27	9.6	0.86	9.6	1.82	0.80
Neu (10^3/µl)	1.0	0.11	1.1	0.20	1.0	0.11	1.2	0.18	2.2	0.83	0.21
Lym (10^3/µl)	7.8	0.86	9.5	0.93	8.1	1.24	7.8	0.71	6.3	1.40	0.35
Mono (10^3/µl)	0.2	0.04	0.4	0.09	0.3	0.09	0.4	0.14	0.8	0.50	0.63
Eos (10^3/µl)	0.1	0.02	0.1	0.01	0.1	0.01	0.1	0.02	0.1	0.01	0.65
Baso (10^3/µl)	0.1	0.02	0.1	0.03	0.2	0.09	0.1	0.02	0.3	0.22	0.65
RBC (10^3/µl)	8.4^{a}	0.10	8.4^{a}	0.11	8.3^{a}	0.20	8.1^{a}	0.14	7.6^{b}	0.10	0.01
HB (g/dl)	15.4^{a}	0.30	14.8^{ab}	0.26	14.5^{ab}	0.36	14.4^{b}	0.32	13.5^{c}	0.22	0.01
PCV (%)	46.9^{a}	0.69	45.8^{ab}	0.72	45.1^{ab}	0.93	44.4^{b}	0.88	41.7^{c}	0.75	0.01
MCV(fl)	55.9	0.89	54.8	0.79	54.2	1.04	54.5	0.45	54.8	0.61	0.64
MCH(pg)	18.4	0.41	17.7	0.20	17.5	0.43	17.7	0.22	17.7	0.20	0.29
MCHC(g/dl)	32.9	0.30	32.4	0.30	32.2	0.22	32.4	0.31	32.4	0.11	0.42
RDW (%)	16.8	0.69	16.0	0.25	15.9	0.50	16.1	0.27	15.5	0.43	0.42
PLT(10^3/µl)	1252.7	66.0	1228.5	34.0	1246.0	74.3	1227.0	83.0	1192.0	51.4	0.97
MPV(fl)	5.3	0.22	5.5	0.25	5.2	0.16	5.3	0.18	5.8	0.19	0.28

[abc] means with different superscripts within the same row differ significantly, SEM = standard error of mean. WBC (White blood cell), Neu (neutrophils), Lym (lymphocytes), Mono (monocytes), Eos (eosinophils), Baso (basophils), RBC (red blood cell), HB (haemoglobin), PCV (packed cell colume), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), RDW(red cell distribution width) PLT (platlets), MPV (Mean platelet volume)

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HPLC METHOD FOR ANALYSIS OF AMINO ACIDS IN SELECTIVE ANIMALS USING *O*-PHTHALDIALDEHYDE PRECOLUMN DERIVATIZATION

R. Jorfi[1], M. Shuhaimi[1, 2*], Y.B. Che Man[1, 3], M.H. Dzulkifly[1, 3] and A.Q. Sazili [4]
[1]Halal Products Research Institute; [2]Faculty of Biotechnology and Biomolecular Science; [3]Faculty of Food Science Technology; [4]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.
*Email: Shuhaimi@biotech.upm.edu.my

Food science is an important area of research that encompasses many disciplines, including the fields of chemistry, biochemistry and microbiology. As a result, the analysis of food has been considered essential in addressing issues including food safety, food composition and quality (1). The determination of food authenticity and the detection of adulteration are major issues in the food industries, and are of concern among consumers. Adulteration mainly happens when supply exceeds market demand and food manufacturers try to fill the gap and gain extra profit by using cheaper substitutes in their products (2).

The detection of pork in various food products, particularly in cooked or processed meat, has been an important subject of study in many countries, especially where serious matters in view of some religions, biological complications and health risks associated with daily intake. Therefore, a rigorous method is an urgent need in order to detect the presence of pork in food (2, 3).

Food products, which are composed of a large variety of small molecules with different chemical properties, have usually been analyzed using chromatographic techniques including HPLC and GC while HPLC is usually well suited for food analysis. Most of the research work reported on this subject has concentrated on the analysis of pork fat and the identification of pork proteins (1, 3). Amino acids exist in food either in the free form or linked together as peptides, polypeptides or proteins. The analytical determination of amino acids in foods has been considered essential as they are vital food nutrients and indicative of protein composition (4).

Amino acids were determined using an Agilent HPLC system, equipped with a quaternary pump delivery system, robotic auto sampler, thermostat column compartment and a Diode array detector. The samples were submitted to automatic pre-column derivatization with OPA-3MPA by programming the robotic auto sampler.

Fifteen amino acids were identified in cow, goat, sheep, chicken and pig with pre-column derivatization (OPA-3MPA) by comparing retention times with those obtained from amino acid standard solutions in our study, with an injection time of 26 min preceded by a derivatization step of which 16 min were for analysis time. The sensitivity of the method was highly enough to estimate amino acid levels injected onto the column with a lower limit. Results of the analysis showed that the meats investigated were distinguishable from each other by measuring the percentage of their corresponding amino acids.

The data taken were analyzed using One-way analysis of variance (ANOVA) to determine the significant differences of amino acids among the various meats studied. Subsequently, the Duncan's New Multiple Range Test (DNMRT) was applied for comparison of means of the amino acids measured in different meats. Principal Component Analysis (PCA) was performed to determine the main directions of variations among the meats. The sample meats were located in a graph based on the first three PCs.

High sensitivity and a lower limit of detection as well as short analysis time was found to be the main advantages of the method used in this study. It can be concluded that this method could be used for fast, simple and cost effective separation of pork from the other meats measured in this study. The finding from this study will serve as a basis in developing a database for monitoring food authentication, especially for Halal authentication purposes.

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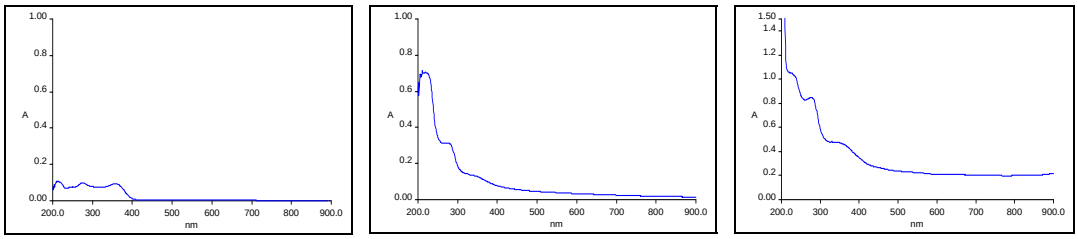
PRODUCTION OF SPECIFIC TETRACYLINE POLYCLONAL ANTIBODIES USING KLH AS CARRIER PROTEIN

B. Siti Noraini*, M.S. Nur Azura, H. Noorshinah, A.R. Gayah, H. Hasanah and S. Faridah
Biotechnology Research Centre, MARDI, Serdang, Selangor.
*Email: ainisnb@mardi.gov.my

Antibiotics are chemical substances that can inhibit the growth of, and even destroy, harmful microorganisms. As the use of antibiotics becomes increasingly important in food production, it has become necessary to take measures to protect consumers from the effects of the antibiotic residues in food [1]. Tetracycline is one of the most popular antibiotics used as veterinary antibiotics in animal husbandry [2]. In Malaysia, the tetracycline maximum residue levels (MRLs) in chicken are set at 100 µg/kg (muscle), 200 µg/kg in eggs, 300µg/kg in liver and 600 µg/kg in kidney. As a consequence of extensive usage of antibiotics, considerable attention has been paid to the potential human health risk due to their carcinogenic potency and possible antibiotic resistance. The aim of this study was to produce polyclonal antibody against tetracycline which could be used in the detection of tetracycline residue.

The experiment was conducted by conjugating the tetracycline to a protein carrier, KLH (Keyhole Limpet Hemocyanin) to generate an immune response for immunization as the tetracycline is a small molecule [3]. The conjugation of tetracycline to a KLH (tetracycline-KLH) was then qualitatively characterized by scanned analysis at 200 to 900 nm using UV-VIS spectrophotometer. The preparation was then used as immunizing antigens and was injected subcutaneously into rabbits. Rabbits were bled for antibody titer determination two weeks after each boost. Blood was collected 30 days after the first immunization. Antibody in serum samples was precipitated with saturated ammonium sulfate, centrifuged, dialyzed and eluted through a protein A sepharose affinity chromatography. Fractions giving the highest absorbance reading for protein at A280 nm were collected and freeze dried for long term storage. Antibody eluted from Protein A sepharose column was determined by indirect ELISA method.

In this study, the conjugation of tetracycline to KLH and the production of antibody polyclonal against tetracycline-KLH are described. Results in Figure 1 show the synthesis of tetracycline-KLH conjugate scanned using a spectrophotometer. Referring to Figure 1 (c), it can be seen that the tetracycline was successfully conjugated with KLH.



(a)Tetra (b) KLH (c) Tetra-KLH conjugate

Figure 1. Synthesis of tetracycline-KLH conjugates when scanned through spectrophotometer wavelength.

Figure 2 shows the results of two peaks obtained from antibody purified using protein A. Peak 1 showed that the antibody had successfully bound with protein A while peak 2 showed that the bound antibody had successfully eluted and was collected via fraction tubes. Antibody titer determination was conducted through ELISA method using tetracycline-KLH conjugate as the antigen (Figure 3). Results showed that the antibody production was successful compared to the preimmune. This suggested that the immunization and purification method used was successful. Antibody production was highest on the third bleed, reaching an absorbance of 1.1 at 0.01 mg/ml concentration. The entire antibody bleeds declined dramatically at dilutions giving greater than 0.0001 mg/ml protein. The optimum and economic concentration of antibody was about 0.00001 mg/ml. In conclusion, the third bleed was shown to be the best titer for sensitivity in this study.

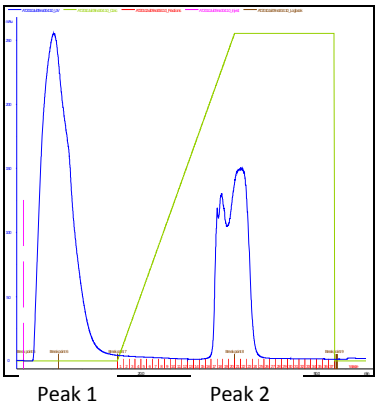


Figure 2: Peaks 1 and 2 after purification of antibody using Protein A affinity chromatography column

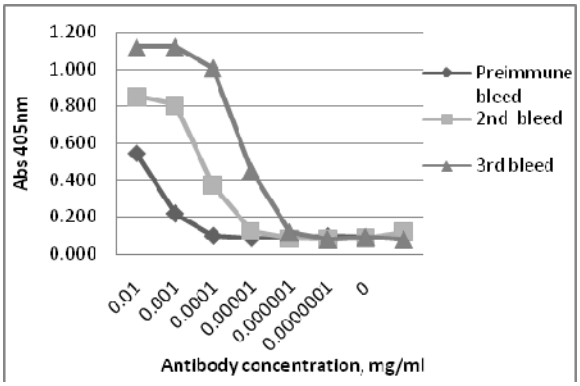


Figure 3: Antibody titer determination

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COMPARISON OF TESTS FOR CROSS REACTIVITY IN CHICKEN MEAT SPIKED WITH *S. TYPHIMURIUM* WITH VARIOUS SPECIES OF ENTEROBACTERIACEAE

R.A. Rahman* and N.A. Masdor
Biotechnology Research Center, MARDI, P.O. Box 12301, GPO Kuala Lumpur, Malaysia
*Email: arafidah@mardi.gov.my

The degree of cross-reactivity reactions among *E. coli*, *V. cholera*, *Shigella* and *L. monocytogenes* in chicken meat spiked with *S. typhimurium* were investigated. *S. typhimurium* is one of the most commonly isolated causative agents of acute gasteroenteritis in human and farm animals. Its precise diagnosis is important for epidemiological investigation. Reports by Pěterferi et al. (1) and Magliulo et al. (2) have described cross reactions between *E. coli* and *Salmonella* antigens. Cross reactions between *E. coli* and *Shigella* were also reported (3). Previously, we have successfully developed a method to detect the contamination of *Salmonella* in foods. This method has been tested on actual meat samples and the results showed that 90% of the samples tested correlated with the validating culturing method. Hence, this study aimed to further investigate the specificity of the kit using cross-reactivity between four species from the Enterobacteriaceae family and *Salmonella* as an indicator.

The study was conducted using refrigerated chicken cuts (thigh, drumsticks, wings and ribs) purchased from a local supermarket. Samples were divided into two sets: Experiment 1 (spiked with *E. coli* and *V. cholera*) and Experiment 2 (spiked with *Shigella* and *L. monocytogenes*). Each set also comprised of positive control (spiked with *S. typhimurium*) and negative control (free from *S. typhimurium*). All of the samples were then subjected to two steps of enrichment by *Salmonella* media followed by tetrathionate broth base. Samples were examined for the presence of *Salmonella* using standard culture method and counter-checked using immuno-dot blot strip developed by MARDI and commercial kits.

E. coli, *V. cholera*, *Shigella* and *L. monocytogenes* were chosen for their similar characteristics to the genus *Salmonella*. It was observed in Experiment 1 that for samples spiked with *E. coli* and *V. cholera*, the results using MARDI's *Salmonella* strip did not correspond with the conventional method and the commercial kit (Table 1). However, for positive and negative samples, the three methods correlated with each other. Concurrently, cross reactions between *Salmonella* and *E. coli* were detected through the positive results indicated by MARDI's *Salmonella* strip. This finding stressed the well-known close relationship between *E. coli* and *Salmonella*. The same trend was also demonstrated in results of Experiment 2 where the three methods employed (*Salmonella* strip by MARDI, *Salmonella* agar and the commercial kit) also did not correlate with each other (Table 2).

Table 1: Cross reactivity results for Experiment 1.

Sample No	Conventional Method (*Salmonella* Agar)			*Salmonella* strip	Commercial kit
	Rep 1	Rep 2	Rep 3		
1	+	+	+	+	-
2	+	+	+	+	-
3	+	+	+	+	-
4	+	+	+	-	-
5	+	+	+	-	-
6	+	+	+	-	-
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-

Sample 1-3 = *E. coli;* Sample = *V. cholera*; Sample 7-8 = Positive samples (spiked with *S. typhimurium);* Sample 9-12 = Negative samples.

Table 2: Cross reactivity results for Experiment 2.

Sample No	Conventional Method (*Salmonella* Agar)			*Salmonella* strip	Commercial kit
	Rep 1	Rep 2	Rep 3		
1	+	+	+	-	-
2	+	+	+	-	-
3	+	+	+	-	-
4	+	+	+	-	-
5	+	+	+	-	-
6	+	+	+	-	-
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-

Sample 1-3 = *Shigella;* Sample 4-6 = *L. monocytogenes*; Sample 7-8 = Positive samples (spiked with *S. typhimurium);* Sample 9-12 = Negative samples.

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EXPRESSION OF PKC-β AND COX-2 GENES IN BOVINE KIDNEY CELLS TREATED WITH PHORBOL ESTERS ISOLATED FROM *Jatropha curcas* Linn. KERNEL MEAL

E. Oskoueian, [1], N. Abdullah[*2,3], S. Ahmad[2], S.W. Zuhainis[1], A.R. Omar[4] and Y.W. Ho[4]

[1]Department of Microbiology, [2]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, [3]Institute of Tropical Agriculture, [4]Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia

*Email: norhani@biotech.upm.edu.my

Jatropha curcas (*J. curcas*) seeds are recognized as a potential source of biofuel. The residue after oil extraction (kernel meal), which is high in protein has the potential as animal protein supplement (1). However, the presence of phorbol esters (PEs) renders the meal unsuitable as an animal feed. Phorbol esters are polycyclic compounds, where two hydroxyl groups on neighboring carbon atoms are esterified by fatty acids (2). Phorbol esters depending on their structures and in particular their functional groups might activate the protein kinase C (PKC) isozymes which regulate different signal transduction pathways (3). Adverse effects were observed when various animals were fed with diets containing PEs [4]. *Jatropha curcas* grown in Malaysia contains PEs [1]. The effects and mechanism of action of PEs from *J. curcas* are still controversial. Therefore, this research was conducted to elucidate the toxicity mechanisms of isolated PEs from *J. curcas* using bovine kidney cells.

Kernels from mature seeds of J. curcas were defatted in a Soxhlet apparatus, using petroleum ether for 16 h. Phorbol esters were isolated from *J. curcas* kernel meal (4). Bovine kidney cells (ATCC:CCL-22) were grown as monolayers in Dulbecco's Modified Eagle Medium. Cells were treated at cytotoxic concentration (CC_{50}) with isolated PEs and phorbol 12-myristate 13-acetate (PMA) as positive control incubated for 12 h. Total RNA was extracted using the RNeasy Mini Kit (Qiagen). RT-PCR was performed using the Quantitect reverse transcription kit (Qiagen). The expressions of protein kinase C-β and COX-2 genes were determined using iQ SYBR Green Supermix Kit (Bio-Rad) by CFX96 Real-Time System (Bio-Rad). Data were normalized to the GAPDH and β-actin as housekeeping genes by CFX Manager software (Bio-Rad) (5). GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA) was used for all the statistical analyses. ANOVA with Dunnett as a post test was used to determine the significant difference among treatments.

Four types of PEs were isolated from *J. curcas* kernel meal and were named as PE1, PE2, PE3 and PE4. Figures 1 and 2 show the fold-changes in the expression levels of PKC-β and COX 2 genes of cells treated with different types of PEs (PE1, PE2, PE3, PE4) isolated from *J. curcas* kernel meal and PMA. Gene expression results showed the significant ($p<0.0001$) upregulation of PKC-β gene with values of 3.0±0.15, 2.86±0.16, 2.5±0.19, 2.5±0.15 and 3.0±0.13 fold changes by cells treated with PE1, PE2, PE3, PE4 and PMA, respectively. Similarly, significant ($p<0.001$) upregulation of COX-2 gene was also observed when the bovine kidney cells were treated with PE1, PE2, PE3, PE4 and PMA with values of 2.9± 0.04, 2.7±0.11, 2.9±0.45, 3.6± 0.30 and 5.0± 0.56 fold changes, respectively. The PEs isolated from *J. curcas* kernel meal behaved similarly to PMA in the expression of PKC-β gene, but the changes were lower for COX-2 gene. It has been reported that oxidative damage and inflammation were induced by PMA on mice skin through over expression of COX-2

gene (6). Chronic inflammation due to prolong exposure to PMA could lead to cancer (7). The present study shows that PEs from *J. curcas* kernel meal induced upregulation of PKC-β and COX-2 genes responsible for inflammation.

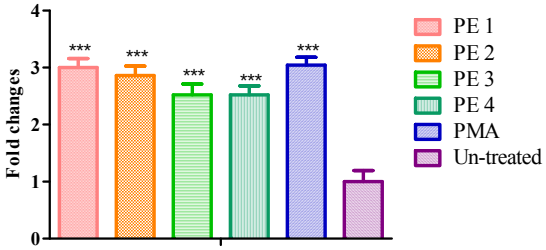


Figure 1: Fold-changes in the expression levels of PKC-β gene

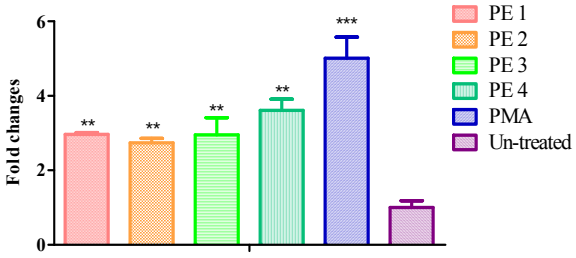


Figure 2: Fold-changes in the expression of COX 2 gene levels COX 2 genes

Note: All values represent mean ± standard error from three independent experiments.
***P<0.001, **P<0.001 indicate significant difference compared to the untreated control.

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DIRECT COMPETITIVE ENZYME IMMUNOASSAY METHODS FOR TETRACYCLINE DETECTION

A.R. Gayah, S. Faridah, R. Hazana, Z. Norzaili, M.S. Nur Azura and I. Zamri
Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute, Serdang, Selangor.
E-mail: gar@mardi.gov.my

Tetracycline is a class of antibiotics with a broad spectrum activity and has been used in the prophylaxis and therapy of human and animal infections. An antibiotic residue in raw chicken is a major concern for food safety and food industries worldwide (1, 2). At present, current methods for tetracycline detection are time and labour intensive. Microbiological inhibition tests are less specific than ELISA due to all antibiotics and all naturally occurring contaminants which possess antibacterial activity can cause growth inhibition of susceptible bacteria. Ideal antibiotic detection systems are often proposed as a microbiological screening, followed by an ELISA test, and final confirmation and quantification using HPLC or GCMS analysis. Therefore, rapid detection of tetracycline is a key to the problems related to health and food safety (3).

This paper describes the development of a direct competitive ELISA method for the detection of tetracycline in chicken sample (muscle). The anti-tetracycline antibody was produced by immunizing rabbit with intra-subcutaneous injection. Lowest antibody titer obtained was 1:51,200. Optimal antibody concentration (0.5 mg mL^{-1}) was used for the construction of the standard calibration curve for tetracycline. Various spiked sample (milk, chicken liver, eggs, wild honey and chicken meat) with various tetracycline concentration (0, 25, 50, 75, 100, 150 and 150 ppb) was used to demonstrate the performance of the developed immunoassay method. The cross-reactivity of the anti-tetracycline antibody with other antibiotics (streptomycine, vancomycine and sulfamethazine) was also tested. Cross-reactivity of the anti-tetracycline antibody with other tetracycline derivatives was also carried out. Storage stability of immunoassay plate coated anti-tetracycline antibody between 6 months and one year duration was also conducted.

Tetracycline in chicken sample (muscle) was detected using direct competitive immunoassay format with highest sensitivity of limit of detection (LOD) down to 75 ppb (Figure 1). Performance of the developed immunoassay methods with various sample spiked with tetracycline showed significant percent of recovery. This method does not show cross-reactivity with samples tested with other antibiotics (streptomycin, vancomycine and chloramphenicol) but could recognize all antibiotic in tetracycline family (Figure 2A and B). This immunoassay plate also had acceptable storage stability with the first 3 month (> 80 % response from the initial response), but the response decrease after one year storage at 4°C (Figure 3). This result indicates that the immunoassay plate had acceptable storage stability and the present immunoassay format is suitable for the determination of tetracycline in animal food sample for routine monitoring. However, it should be noted, that no stabilizing chemistries where used in these experiments. The use of sugar alcohol to stabilize the ELISA reagents on the plate surface can be considered for future research and commercialisation.

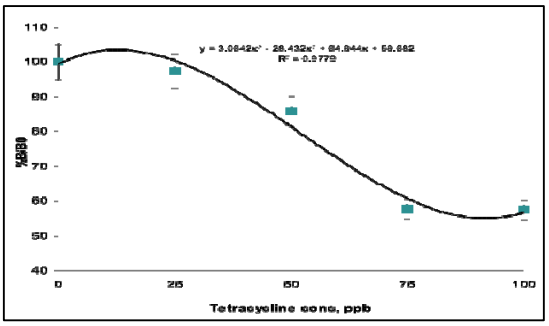


Figure 1: Tetracycline standard curve with the LOD at 50 ppb

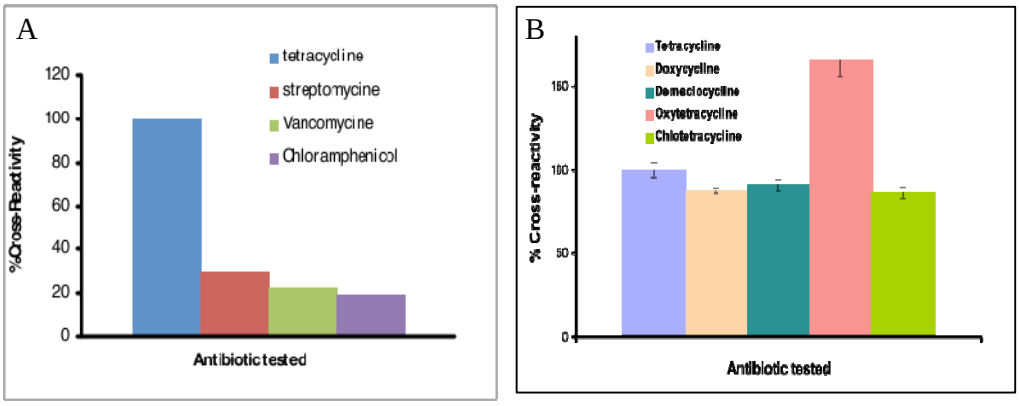


Figure 2: Cross-reactivity of anti-tetracycline antibody with others antibiotic (A), with tetracycline derivatives (B)

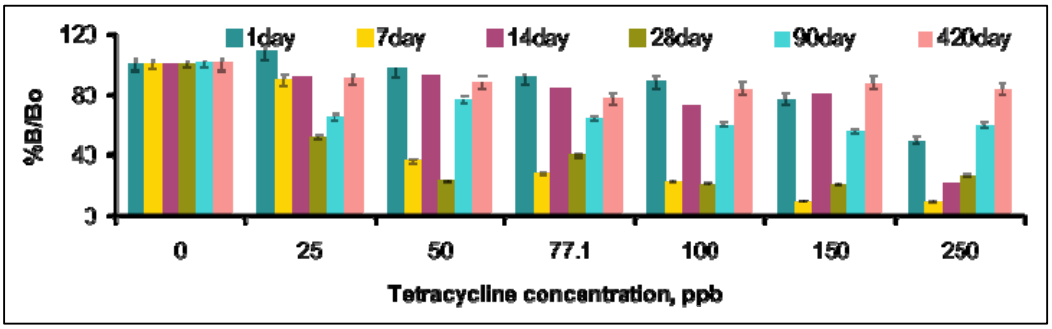


Figure 3: Storage stability of immunoassay plate for a period of 3-12 months at 4 °C.

Based on this research finding, the potential of immunoassay methods based on antibody-antigen binding is suitable for rapid and sensitive assay for antibiotic detection and will be potential for used as a sensor platform in the analysis of tetracycline antibiotic using carbon screen-printed sensor and miniaturisation instrument (potentiostat) for in *situ* and on-site food monitoring application.

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INITIAL DEVELOPMENT OF RAPID IMMUNO - LATERAL FLOW STRIP FOR TETRACYCLINE DETECTION

S. Faridah *, R. Hazana, A.P. Nurul Hidayah, M.S. Nur Azura, A.R. Gayah, Z. Norzaili and I. Zamri

Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute, Serdang, Selangor

*E-mail: faridahs@mardi.gov.my

The tetracycline is a class of antibiotics with a broad spectrum activity and has been used in the prophylaxis and therapy of human and animal infections. An antibiotic residue in raw chicken is a major concern for food safety and food industries worldwide (1, 2) At present many of the currently used methods for tetracycline detection using Microbiological inhibition tests are time and labour intensive and also less specific due to growth inhibition of susceptible bacteria. Detection and quantification using HPLC or GCMS analysis is reliable with high percentage (99% - 100%) but it was very expensive and labour cost. Therefore, rapid detection based on immuno-chromatographic assays of antibody specific with an antibiotic with the application of gold nano-particle to enhance the detection sensitivity of tetracycline assay is the problems solution with our antibiotic monitoring system in this country[3].

A rapid immuno-chromatographic assays based on lateral flow concept is designed to detect an antibiotic analyte in direct competitive configuration which possible to obtain the test results in 5-10 minute after applying a sample. The assay is known to be sensitive for an antibiotic detection with the detection limit at 100 ppb for tetracycline antibiotic. The schematic diagram of the test strip (Figure 1) described the detection principle started with the antibiotic reacts with detector antibody- nano gold when the liquid sample was dropped on the sample releasing pad. A single band appeared at control line indicate positive antibiotic in samples, while negetive antibiotic will shows two band at control and test line. Chicken samples from muscle and organs with simple extraction method can be applied in this test strip.

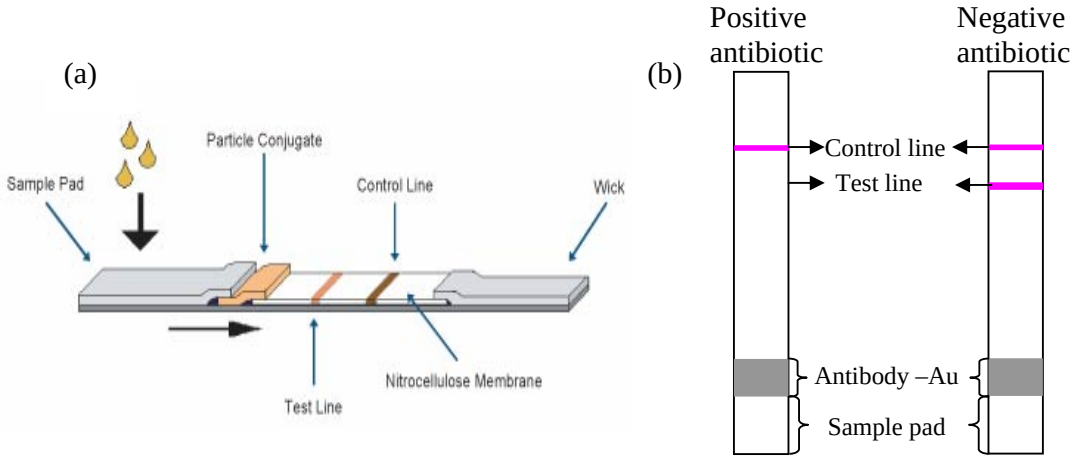
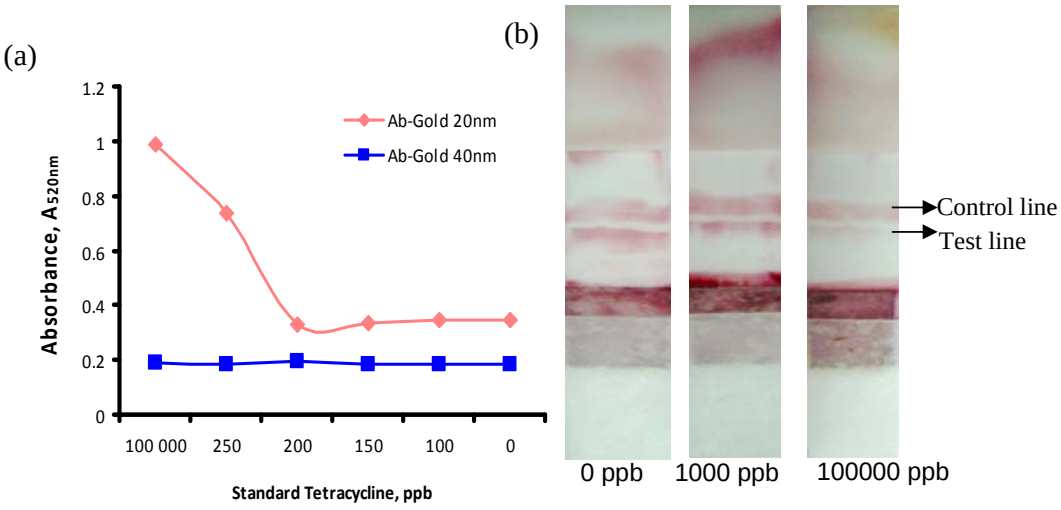


Figure 1: (a) Immuno-lateral flow strip principle (b) Positive and negative results on test strip when applying the sample containing tetracycline antibiotic

In this study, the use of different diameter colloidal gold particles, 5 nm, 10 nm, 20 nm and 40 nm, for the production of the antibody conjugate, has been explored. The optimal size of colloidal gold that is suitable to be conjugated with IgG in immuno-

lateral flow strip development is 20 nm. This size provides the most stable conjugate form (Figure 2 a, b).



(c)

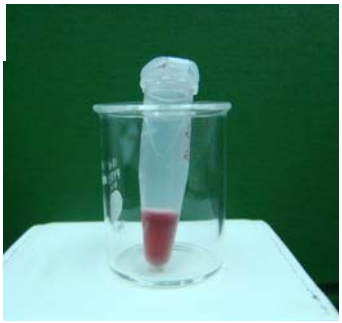


Figure 2: (a) Comparison of binding activity for antibody conjugated colloidal gold (20 and 40 nm) in immunoassay at different wavelengths (b) Test strip labeling with colloidal gold generates changes in color density, in accordance with the antibiotic concentration. (c) The color of the colloidal gold conjugates

With this research finding, gold nano-particle can be used to replace enzyme labeled -antibody in normal immunoassay system. The binding of nano-gold-antibody is non-covalent bonding which not changing the antibody activity and it was more stable and easy to use. Larger gold particles may yield higher signal, but the higher radius of the particles can pose some stearic limitations to the assay.

As an initial platform of the lateral flow disposable strip for antibiotic detection, this method is able to detect tetracycline antibiotic at 100 ppb for the lowest detection. This finding is very useful as an initial development of disposable inexpensive diagnostic strip for on site monitoring of antibiotic residues in poultry industries especially at the processing plan

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IN VITRO DEVELOPMENT OF IVF-DERIVED HYBRID EMBRYOS USING BOVINE OOCYTES AND *BOS JAVANICUS* SPERM

Mojtaba Dashtizad[1], H. Wahid[1*], Y. Rosnina[1], M. Daliri[2], H. Hajarian[1], O. Abas Mazni[3]

[1]Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; [2]National Institute of Genetic Engineering and Biotechnology (NIGEB), 14965 Tehran, Iran; [3]Agro-Biotechnology Institute (ABI), 43400 Serdang, Selangor, Malaysia

[*]Email: wahid@vet.upm.edu.my

The demand for livestock products is continue to rise due to world's population growth. In the tropical climate, local cattle do not have suitable meat production performance to be use in the feedlot production system (1). Heat stress as well as high temperature and humidity, adversely affects livestock production. On the other hand, *Bos javanicus* (Banteng) is a vigorous native wild bovine which is able to tolerate variety of climate, from warm and wet to hot and dry season (2) and thrive in the tropical climate. However, it has been classified as a critically endangered species. Therefore, hybridization of domestic cattle and such local wild bovine may be an alternative way to improve livestock production system in Malaysia. Advanced reproductive biotechniques such as artificial insemination (AI), *in vitro* maturation, *in vitro* fertilization, and gamete or embryo manipulation are becoming common ways to control hybridization and improvement of desirable characteristics and livestock production of both captive and natural population via heterosis (3). This study was carried out to investigate the ability of banteng sperm to fertilized *in vitro* matured bovine oocytes following IVF.

Cumulus-oocyte complexes were recovered from slaughterhouse derived bovine ovaries. A total of 1392 immature oocytes were cultured in maturation medium consist of Hepes-buffered medium 199 supplemented with 10% fetal bovine serum, 1 µg/ml estradiol and 50 µg/ml gentamycin. After maturation, representative number of oocytes were subsequently fertilized and cultured *in vitro*. *In vitro* matured bovine oocytes were subjected to *in vitro* fertilization (IVF) procedure using either homologous bovine or heterologous banteng frozen-thawed sperm.

Our finding indicated that IVF-derived hybrid embryos could successfully progress to the transferable stage embryos. As shown in table 1, inseminated bovine oocytes with both homogenous (cattle) and heterogeneous (banteng) sperm were successfully developed to the transferable stage embryos. A total of 89.05% (626/703) cattle × cattle and 51.94% (358/689) banteng × cattle oocytes were cleaved. The proportion of purebred IVF-derived morula, blastocyst and expanded blastocyst were notably superior to the corresponding stage in hybrid IVF-derived embryos (Table 1).

In conclusion, *in vitro* mass production of *Bos javanicus* hybrids embryos that successfully achieved in this study can be considered as the first step to introduce a novel source of meat for the future.

Table 1: Comparison of embryo development after fertilization of *in vitro* matured bovine oocytes with either bovine or *Bos javanicus* (Banteng) sperm following IVF

Source of sperm	No. of inseminated oocytes	Type of embryo	Developmental stage achieved per oocytes			
			Cleavage % (n)	Morula % (n)	Blastocyst% (n)	Expanded blastocyst% (n)
Bovine (Control)	703	Purebred	89.05[a] (626/70)	68.77[a] (484/70)	40.15[a] (282/703)	17.38[a] (122/703)
Banteng	689	Hybrid	51.94[b] (358/689)	38.88[b] (268/689)	10.17[b] (70/689)	5.96[b] (41/689)

Data were pooled from 10 replicates. [a, b] Values with different superscripts in the same column are significantly different ($P<0.05$; ANOVA and Duncan's test).

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