ISSN 0216-6461
e-ISSN 2354-6832
Terakreditasi LIPI
Sertifikat Nomor: 644/AU3/P2MI-LIPI/07/2015

WARTAZOA

Buletin Ilmu Peternakan dan Kesehatan Hewan Indonesia
Indonesian Bulletin of Animal and Veterinary Sciences

Volume 25
Nomor 4
Desember 2015

PUSAT PENELITIAN DAN PENGEMBANGAN PETERNAKAN
BADAN PENELITIAN DAN PENGEMBANGAN PERTANIAN
KEMENTERIAN PERTANIAN

WARTAZOA

Buletin Ilmu Peternakan dan Kesehatan Hewan Indonesia

Volume 25 Nomor 4 Tahun 2015

ISSN 0216-6461
e-ISSN 2354-6832

Terakreditasi LIPI
Sertifikat Nomor 644/AU3/P2MI-LIPI/07/2015
(SK Kepala LIPI No. 818/E/2015)

Diterbitkan oleh:
Pusat Penelitian dan Pengembangan Peternakan
Badan Penelitian dan Pengembangan Pertanian
bekerjasama dengan
Ikatan Sarjana Peternakan Indonesia

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Wartazoa diterbitkan empat kali dalam setahun pada bulan Maret, Juni, September dan Desember

KATA PENGANTAR

Industri-industri manufaktur utama Indonesia berbasis pertanian harus lebih responsif terhadap tantangan strategis baik domestik maupun global, termasuk masyarakat ekonomi ASEAN (MEA) yang akan efektif dilaksanakan pada akhir tahun 2015. Industri susu di Indonesia adalah bisnis yang sangat potensial untuk berperan signifikan di pasar ASEAN berdasarkan populasi sapi perah, sumber pakan dan jumlah peternak yang ada. Peluang bisnis industri sapi perah skala UMKM sangat strategis, karena dapat memperpendek rantai pasok susu segar. Oleh sebab itu, dukungan pemerintah pusat dan daerah yang berkelanjutan sangat diperlukan untuk menjaga kesinambungan kemitraan UMKM dengan industri pengolahan susu.

Limbah pertanian merupakan biomassa yang berpotensi menggantikan sebagian bahan pakan konvensional walaupun terdapat kendala dalam pemanfaatannya yaitu berupa serat kasar yang tinggi, serta protein dan karoten yang rendah. Upaya untuk memperbaiki kualitas bahan pakan dapat dilakukan dengan teknologi maju seperti fermentasi menggunakan kapang karotenogenik *Neurospora.* Pemberian pakan berteknologi maju dapat meningkatkan produktivitas dan kualitas produk ternak dengan biaya yang lebih efisien.

Pada bidang kesehatan hewan, teknologi transkripsi dan translasi *in vitro* dapat mengantisipasi timbulnya penyakit-penyakit baru. Teknik ini secara cepat dan tepat berguna untuk skrining vaksin maupun menghasilkan vaksin hewan baru. Partikel nano sebagai partikel dispersi berukuran sangat kecil yang dilapisi dengan polimer dapat digunakan sebagai komponen pembawa hormon yang potensial karena kemampuannya dalam meningkatkan stabilitas hormon dan melepaskan hormon secara terkontrol dalam waktu lama di dalam tubuh ternak. Pemberian hormon yang dikemas dalam partikel nano mulai berkembang dalam beberapa tahun terakhir ini memberi keuntungan karena lebih efisien dan lebih mudah untuk diaplikasikan.

Dewan penyunting menyampaikan terima kasih kepada para penulis, mitra bestari dan semua yang terlibat dalam publikasi ini.

Bogor, Desember 2015

Ketua Dewan Penyunting

WARTAZOA

Buletin Ilmu Peternakan dan Kesehatan Hewan Indonesia

Volume 25 Nomor 4 (Desember 2015)

ISSN 0216-6461
e-ISSN 2354-6832

DAFTAR ISI Halaman

WARTAZOA

ISSN 0216-6461 Date of issue 2015-12-31

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UDC 619
Hartawan R (IRCVS, Bogor)
Dharmayanti NLPI (IRCVS, Bogor)
Identification and Characterization of Marek's Disease Virus Serotype 1 Using Molecular Approaches (Orig. Ind.)
Wartazoa March 2015, Vol. 25 No. 1, p. 1-14

Marek's disease is an important disease in the commercial poultry farm and causes a significant economical loss. The disease is characterized by syndrome of paralysis and neoplastic formation in various organs and tissues in the host. The etiological agent is Marek's disease virus serotype 1 (MDV-1). Eventhough the outbreaks in the field are well controlled by vaccination, several cases in the vaccinated flocks indicating virus evolution into more pathogenic strains. Therefore, monitoring of the disease circumstance in the field is indispensable for guiding better policies in disease controlling program. This paper describes several molecular methods that have been developed for identification and characterization of MDV-1. The identification and characterization of newly found virus strain in the field can be done by *in vivo* challenge test which is a conventional method especially to determine pathogenecity. However, this method requires several stages with time consuming procedures. The development of alternative methods for identification and characterization of MDV-1 viruses has been conducted mainly using molecular biology approach. Several molecular methods give satisfying result and have been implemented in both laboratory and field condition.

(Author)

Key words: Marek's disease virus serotype 1, identification, characterization, molecular

UDC 636.597
Susanti T (IRIAP, Bogor)
Prolactin as a Candidate Gene Controlling Molting and Egg Production of Duck (Orig. Ind.)
Wartazoa March 2015, Vol. 25 No. 1, p. 23-28

Incidence of molting is a crucial problem in the local ducks that need to be handled from many aspects including genetic aspect. Handling of molting genetically can be done quickly and accurately when the control genes have been found. The search for marker genes of molting can be conducted in poultry through broodiness naturally, because its physiological processes are related to the continuity of egg production. This paper describes the mechanism of molting, the relationship of molting with prolactin hormone and the association of prolactin gene polymorphism with molting and egg production. Molting and egg production were influenced by the prolactin hormone, that may be controlled by the prolactin gene. High concentration of prolactin hormone will inhibit the function of pituitary gland, decreasing production of gonadotrophin hormone (follicle stimulating hormone and luteinizing hormone) hence ovulation ceased. This will stop egg production and at the same time molting proccess occurred.

(Author)

Key words: Prolactin gene, molting, egg production, duck

UDC 636.58.033
Ulupi N (Bogor University of Agricultural, Bogor)
Sumantri C (Bogor University of Agricultural, Bogor)
The Role of Triglyceride Lipase, Fatty Acid Synthase and Fatty Acid Binding Protein Family Genes on Fat Metabolism of Broiler Chickens (Orig. Ind.)
Wartazoa March 2015, Vol. 25 No. 1, p. 15-22

Broiler chicken has high fat content, especially abdominal and subcutan fat which reduced carcass quality and efficiency of feed energy. Genetic approach could be potentially applied to reduce high abdominal and intramuscular fat in broiler chicken through the selection program at gene level related to fat metabolism. This paper describes the metabolism and biosynthesis of body fat and the role of its controlled genes. Fat synthesis is controlled by feed quality and metabolism and biosynthesis process occurred in liver. These processes are controlled by many family genes, but certain genes have dominant role in the process; those are triglyceride lipase genes, fatty acid synthase genes and fatty acid binding protein genes. Expression of fatty acid synthase genes has positive correlation with fat content in liver and intramuscular. Expression of fatty acid binding protein genes was related to the increased abdominal fat deposit. These genes are polymorphic, so that they can be used as a genetic marker in selection to optimize feed efficiency, to minimize abdominal fat and to increase economic value of broiler chicken.

(Author)

Key words: Fat, triglyceride lipase genes, fatty acid synthase genes, fatty acid binding protein genes

UDC 619:591.2
Dharmayanti NLPI (IRCVS, Bogor)
Sendow I (IRCVS, Bogor)
Awareness of Ebola: An Exotic Zoonotic Disease (Orig. Ind.)
Wartazoa March 2015, Vol. 25 No. 1, p. 29-38

Filovirus including Ebola and Marburg hemorrhagic fever is a zoonotic disease that characterised by immune suppression and systemic inflammatory response causing impairment of the vascular and immune systems. It is leading to multiorgan failures with mortality varies from 50-90% in human and primate. The Ebola virus is currently divided into five species, namely *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SEBOV), *Tai Forest ebolavirus*, *Reston ebolavirus* (REBOV) and *Bundibugyo ebolavirus*. Geographical distribution of Ebola virus in the Afrotropics region is mainly in the rainforests of Central and West Africa, while REBOV was detected in the Philippines. Bats are suspected as reservoir host of the virus. Recently, Ebola cases had been reported in endemic areas in Africa and then distributed to other countries which were not endemic through human travellers. Ebola virus is also potentially used as a biological weapon, so Ebola virus becomes public health concern. This paper describes the characters of Ebola virus, its clinical signs, transmission and threat as an exotic disease in Indonesia. By understanding the disease, the emergence of Ebola virus in Indonesia can be anticipated quickly.

(Author)

Key words: Ebola virus, exotic, pathogen

UDC 636.4
Soewandi BDP (IRIAP, Bogor)
Talib C (IRIAP, Bogor)
Development of Local Pig in Indonesia (Orig. Ind.)
Wartazoa March 2015, Vol. 25 No. 1, p. 39-46

Indonesia is a country that has the largest swine germplasm in the world and having five out of eight species, but the population of local pig has been decreasing toward extinction. This paper describes characteristic of local pig and factors that cause endangered of germplasm and strategy to prevent the declined population. One of the factors that causing decreased of local pig population is due to its lower productivity. Government policies for the development of local pigs have not been planned yet because of the socio-cultural barriers. Therefore, establishment of the genetic resource conservation for local pig area is required. In addition, local pig preservation activities can be integrated with the promotion of cultural heritage and local traditions. Development strategy should be planned to increase local pig value, including (1) Build a genetic resource conservation area in the outer islands in Indonesia for wild pigs; (2) Preserving local pigs to develop local pig farms by community; and (3) Integrating maintenance of local pig farm with cultural activities through the establishment of village/tourist area.

(Author)

Key words: Local pig, characteristic, development

UDC 619
Suartini IGAA (Udayana University, Bali)
Sendow I (IRCVS, Bogor)
The Prospect of Immunoglobulin Y for Therapy of Canine Parvovirus Infection in Dogs (Orig. Ind.)
Wartazoa June 2015, Vol. 25 No. 2, p. 55-64

Canine parvovirus (CPV) is a highly infectious virus. The virus causes death in dogs worldwide. The mortality rate due to infection of CPV in dog reaches 91%. Prevention of CPV infection in puppies has been done by vaccination which is effectively proven. Protective mechanisms of maternal antibodies contribute to the failure of vaccination. Highly stable characteristics of parvovirus enable the virus still exist in the environment. Various therapies are performed only to suppress the clinical symptoms but can not reduce puppy mortalities. This review discusses CPV alternative therapy and the advantages using immunoglobulin Y (IgY) specific antibodies isolated from chicken egg yolk. Immunoglobulin Y will neutralize the virus, so it can not infect host cells. Intravenous IgY therapy has shown to suppress the spread of CPV infection and prevent death.

(Author)

Key words: Parvovirus, canine, immunotherapy, immunoglobulin Y

UDC 633.2
Nurhayati DP (IRIAP, Bogor)
Tiesnamurti B (ICARD, Bogor)
Adinata Y (IRIAP, Bogor)
Availability of Forage Under Oil Palm Plantation for Cattle Grazing (Orig. Ind.)
Wartazoa March 2015, Vol. 25 No. 1, p. 47-54

Increasing rate of oil palm plantation in Indonesia since 2008-2011 was 6.92%, that increased from 7,363,703 to 7,873,384 ha. Vegetation grown in the area of oil palm plantation is weed for its main crop. There is potential source of oil palm plantation area for livestock industry. Oil palm-cattle integration system is well known and it has been applied in many oil palm plantations, by the use of waste from oil palm plantation, oil palm by-product, the fronds for feed and feces from cattle as organic fertilizer for the plant. Management of oil palm plantation, including plant maintainance, weeding, providing organic and chemical fertilizer is costly. Grazing system under oil palm would minimize cost problem and oil palm production input can be reduced. One of the systems in oil palm-cattle integration that prospective to be developed is grazing by rotation system. Types of plants under oil palm plantation consist of grasses, legumes, other narrow and broad leaves; some are palatable and some are unpalatable or toxic for cattle. Species of vegetation under oil palm vary among plantations depending on the age of oil palm plant. Introduction of superior forage into oil palm plantation is promising effort to increase the production and quality of feed. Carrying capacity for cattle varies among the oil palm plantation and depends on vegetation under oil palm plantation and age of oil palm. Studies showed that integration oil palm-livestock by grazing system has been proven economically feasible.

(Author)

Key words: Plantation, oil palm, forage, cattle, grazing

UDC 619
Nurhayati IS (ICARD, Bogor)
Martindah E (IRCVS, Bogor)
Controlling Subclinical Mastitis by Antibiotic Application During Dry Period of Dairy Cow (Orig. Ind.)
Wartazoa June 2015, Vol. 25 No. 2, p. 65-74

Prevention of mastitis is essential, as one of the efforts to control disease in dairy cow. Dry period has implications to understand the mastitis and its control strategies. The udder is very susceptible to be infected both at the beginning and towards the end of dry period. This is linked to physiological changes in udder. Treatment with antibiotics during the dry period can reduce new infection about 82% and has several advantages. The success rate of subclinical mastitis treatment is much higher (80-90%) compared to the treatment during lactation (30-40%); the doses of antibiotic can be higher and safer, due to its retention time in udder becomes longer; the risk of antibiotic contamination in milk can be avoided because the udder is not milked. Antibiotic application during dry period is the best way to treat subclinical and chronic mastitis. Treatment during dry period is a specific mastitis control for intramammary infection to avoid economic losses.

(Author)

Key words: Antibiotic, dry period, subclinical mastitis

UDC 619
Tarigan S (IRCVS, Bogor)
Subclinical Infection by Avian Influenza H5N1 Virus in Vaccinated Poultry (Orig. Ind.)
Wartazoa June 2015, Vol. 25 No. 2, p. 75-84

Highly Pathogenic Avian Influenza (HPAI) H5N1 is endemic in Indonesia especially in unvaccinated sector-4 poultry. Considering that vaccination against influenza viruses does not induce sterilizing immunity and the source of infection is prevalent around the vaccinated farms, infection in the commercial layers and breeders may be common. Because infection in vaccinated birds is usually subclinical, its presence is unnoticable. The virus in such farms may be circulated persistently and become the source of infection to the surrounding areas. The test, Differentiation Infected from Vaccinated Animals (DIVA) that can be used to identify subclinically infected farms is not available yet in Indonesia. Observation on sentinel chicken among vaccinated birds is a sensitive and accurate method but unsafe for HPAI. The DIVA method based on heterologous neuraminidase has been successfully used in Italy, but it is difficult to be applied in Indonesia. The DIVA method based on *Ectodomain* protein M2 virus Influenza (M2e) uses antibody against M2e as infection marker and does not limit the subtype of vaccine used. This method is potential to be used in Indonesia because the M2e is very conserved across all avian influenza viruses and has high proportion of post-infected seroconverted birds.

(Author)

Key words: H5N1, DIVA method, heterologous neuraminidase, M2e, vaccination

UDC 636.5
Ilham N (ICASEP, Bogor)
Government Policies on Small Scale Poultry Business and Environmental Health in Indonesia (Orig. Ind.)
Wartazoa June 2015, Vol. 25 No. 2, p. 95-105

The government paid great attention to develop small-scale poultry business, to reduce poverty alleviation and increase employment opportunities. The government has established various policies to encourage the growth of poultry production cluster (PPC) in rural areas. However, the fact shows that these policies have not been able to solve the problems. Small-scale poultry business is particularly vulnerable to economic changes, including animal diseases. The economic crisis of 1997-1998 and avian influenza outbreaks in 2004-2006 had caused most of small-scale enterprises collapsed. Government policies to develop small scale poultry business which is environmental friendly are required so its existence does not disturb the public. Since 2006, the government has established various policies, ie. Village Poultry Farming (VPF) and compartment structuring. Based on evaluation and existing cases, the results have no meet the expectation yet, due to lack of sustain supervision. On the other hand, small scale poultry business has been set up on PPC's under partnerships with companies. The government is expected to continue VPF program and should pay attention to the development of PPC that basically has been accepted by rural communities.

(Author)

Key words: Government policies, poultry, small scale, environmental health

UDC 637.1:636.2.034
Priyono (ICARD, Bogor)
Priyanti A (ICARD, Bogor)
Strengthening Dairy Cooperative Through National Development of Livestock Region (Orig. Ind.)
Wartazoa June 2015, Vol. 25 No. 2, p. 85-94

Establishment of dairy cattle development region needs to be conducted in accordance with the national dairy industry development plan. Dairy cattle regions have been designed and equipped with infrastructure supplies, supporting facilities, technologies, finance, processing, marketing, institutional and human resources. Dairy cooperative is one of the marketing channels of milk and milk products which have strategic roles to support the national dairy industry. Collaborations between dairy cooperatives and smallholder farmers within a district region have to be done based on agricultural ecosystems, agribusiness system, integrated farming and participatory approach. This may improve dairy cooperatives as an independent and competitive institution. Strengthening dairy cooperatives in national region dairy cattle was carried out through institutional inventory and dairy cooperatives performance; requirement of capital access, market and networks as well as education and managerial training; certification and accreditation feasibility analysis and information and technology utilization. Establishment of emerging dairy cooperatives towards small and micro enterprises is carried out by directing them to establish cooperatives which have legal certainty and business development opportunities. The impact of strengthening dairy cooperative may support dairy cattle development through increase population and milk production. Sustainable dairy cattle development needs to be supported by regional and national government policies.

(Author)

Key words: Dairy cooperatives, animal husbandry district development, dairy

UDC 636.087:591.53
Anggraeny YN (BCATRES, Grati)
Soetanto H (Brawijaya University, Malang)
Kusmartono (Brawijaya University, Malang)
Hartutik (Brawijaya University, Malang)
Synchronization of Protein and Energy Supply in the Rumen to Improve Low Quality Feed Efficiency (Orig. Ind.)
Wartazoa September 2015, Vol. 25 No. 3, p. 107-116

Agricultural by-products which can be used as source of roughage, have some limitations as they contain low crude protein and low dissolved organic material and high crude fiber. Synchronization of nutrients through supplementation can provide a positive effect on microbial protein synthesis, especially on ruminants fed low quality forage. Contribution of protein from rumen microbes is essential for feed management based on agricultural by product. Microbial protein can supply 70-100% of the total protein available for ruminants fed low quality feed. Microbial protein has amino acid profile which is ideal to meet ruminant's requirement. This paper describes synchronization of protein and energy supply in the rument that has been applied by several countries. Application of this synchronization in Indonesia is still limited on: (1) Arranging the use of feedstuffs through the ratio of forage and concentrate; (2) Supplementation of protein and energy sources; and (3) Feeding frequency regulation. The application of synchronization through the use of feed ingredients based on degradation level and its index value is still limited due to lack of data on protein and energy degradation of feed ingredients used in Indonesia. Therefore, the information on the degradation value of protein and energy of feed ingredients in Indonesia is necessary in order to optimize the use of low quality feed ingredients.

(Author)

Key words: Nutrient synchronization, microbial protein, fiber source, agricultural by-product

UDC 664.786.8
Tirajoh S (Papua AIAT, Jayapura)
Utilization of Foxtail Millet (*Setaria italica*) From Papua as an Alternative Feedstuff to Substitute Corn (Orig. Ind.)
Wartazoa September 2015, Vol. 25 No. 3, p. 117-124

Papua foxtail millet (*Setaria italica*) is a plant which has been used as a source of carbohydrate, but it has not been used optimally. High demand in consuming corn as poultry feed provides an opportunity for Papua foxtail millet to be used as a substitute for corn in feed. Evaluation of nutritive values and antinutrient shows that Papua foxtail millet is potential to be used as feed stuff. Studies on cultivation technology, evaluation of the nutritive values and antinutrient and its benefits as an alternative feed are relatively limited. The results show that the Papua foxtail millet contains dry matter (88.37%), ash (0.86%), protein (12.07%), fat (2.76%), crude fiber (1.93%), metabolizable energy (3,139 kcal/kg) and anti-nutritional factors (3.07% of phytate and 0.01% of tannins). Several studies reported that the use of Papua foxtail millet at various levels (25-100%) in feed, can substitute corn and give a positive response on consumption, daily weight gain, feed conversion, carcass composition and percentages and egg production. It can be concluded that the Papua foxtail millet can be used as a corn substitution in poultry feed.

(Author)

Key words: Papua foxtail millet, nutritive value, antinutrition, alternative feed

UDC 619:636.5
Hewajuli DA (IRCVS, Bogor)
Dharmayanti NLPI (IRCVS, Bogor)
The Role of Non-specific and Specific Immune Systems in Poultry Against Newcastle Disease (Orig. Ind.)
Wartazoa September 2015, Vol. 25 No. 3, p. 135-146

Newcastle disease (ND) is caused by avian paramyxovirus-1 which belong to *Avulavirus* genus and Paramyxoviridae family. The birds have abnormalities in humoral (bursa fabricius) and cellular (thymus and spleen) lymphoid organs. Lesions decrease the immune system. Immune system consists of non-specific and specific immune systems. The main components of non-specific immunity are physical and chemical barrier (feather and skin or mucosa), phagocytic cells (macrophages and natural killer), protein complement and the mediator of inflammation and cytokines. Interferons (IFNs) belong to a group of cytokines that play a major role in the nonspecific or innate (natural) immunity. The virulent ND virus encodes protein of V gene can be suppressed IFN type I. This leads to non-specific immune system fail to respond to the virulent strains resulting in severe pathogenicity. The defense mechanism of the host is replaced by specific immunity (adaptive immunity) when natural immunity fails to overcome the infection. The specific immune system consists of humoral mediated immunity (HMI) and cell-mediated immunity (CMI). The cells of immune system that react specifically with the antigen are B lymphocytes producing the antibodies, T lymphocytes that regulate the synthesis of antibodies and T cells as effector or the direct cytotoxic cells. Both non-specific and specific immunities are complementary against the invasion of ND virus in the birds. The objective of this article is to discuss the role of non specific and specific immune system in ND.

(Author)

Key words: Newcastle disease, lymphoid organs, non-specific, specific immunity

UDC 636.58.033
Hidayat C (IRIAP, Bogor)
Reducing Abdominal Fat Deposition in Broiler Through Feeding Management (Orig. Ind.)
Wartazoa September 2015, Vol. 25 No. 3, p. 125-134

Abdominal fat in broiler carcass is considered as a waste and its existence reduces the carcass quality. Abdominal fat deposition is affected by several factors such as genetic, nutrition, feed, sex, age and environment. Reducing abdominal fat deposition can be carried out by regulating the nutrient intake to ensure that no excessive nutrient is consumed. Nutrition effects to reduce abdominal fat deposition are associated with nutrient concentration of ration and quantity of daily feed intake. Daily nutrient intake can be limited, especially through restricted feeding. It is concluded that an appropriate feeding management can reduce abdominal fat deposition in broiler.

(Author)

Key words: Broiler, abdominal fat, feed

UDC 636.21
Matondang RH (ICARD, Bogor)
Talib C (IRIAP, Bogor)
Integrated Bali Cattle Development Model Under Oil Palm Plantation (Orig. Ind.)
Wartazoa September 2015, Vol. 25 No. 3, p. 147-157

Bali cattle have several advantages such as high fertility and carcass percentage, easy adaptation to the new environment as well. Bali cattle productivity has not been optimal yet. This is due to one of the limitation of feed resources, decreasing of grazing and agricultural land. The aim of this paper is to describe Bali cattle development integrated with oil palm plantations, which is expected to improve productivity and increase Bali cattle population. This integration model is carried out by raising Bali cattle under oil palm plantation through nucleus estate scheme model or individual farmers estates business. Some of Bali cattle raising systems have been applied in the integration of palm plantation-Bali cattle. One of the intensive systems can increase daily weight gain of 0.8 kg/head, calfcrop of 35% per year and has the potency for industrial development of feed and organic fertilizer. In the semi-intensive system, it can improve the production of oil palm fruit bunches (PFB) more than 10%, increase harvested-crop area to 15 ha/farmer and reduce the amount of inorganic fertilizer. The extensive system can produce calfcrop ≥70%, improve ≥30% of PFB, increase business scale ≥13 cows/farmer and reduce weeding costs ≥16%. Integrated Bali cattle development may provide positive added value for both, palm oil business and cattle business.

(Author)

Key words: Model, integration, Bali cattle, oil palm plantations

UDC 637.1
Priyanti A (ICARD, Bogor)
Soedjana TD (ICARD, Bogor)
Indonesian Dairy Industry Perspective within the ASEAN Economic Community (Orig. Eng.)
Wartazoa December 2015, Vol. 25 No. 4, p. 159 - 170

Many of Indonesian main manufacturing industries based on agriculture should be more responsive to the challenging domestic and global strategic environment, including the newly emerging ASEAN economic community (AEC) which will be effectively implemented by the end of 2015. Dairy industry in Indonesia is a very potential business to play significant role in the ASEAN market based on the existing dairy population, feed resources and the number of dairy farmers. Recently, small and medium enterprise (SME) model in Java has been developed for processing milk. They provide higher farm gate prices under partnership agreement which created a good benchmark and business model for the future dairy industry to adopt. This new attractive business environment gives higher return to the farmers as opposed to the tradition of paying low farm gate milk prices. As feed represents 80% of the total production cost, special attention must be given to land availability to increase feed supply in terms of quality and quantity. Consequently, sustainable support from both central and local government is very critical to keep the partnership model between farmers and the SME milk processing. It also opens new opportunity to increase the linkage more closely between producers and milk processing plants.

(Author)
Key words: SME dairy industry, Indonesia, ASEAN economic community

UDC 619
Ali M (Mataram University, Mataram)
Efforts to Develop Rapid Technology of *In Vitro* Transcription and Translation in Vaccine Synthesis in Indonesia (Orig. Ind.)
Wartazoa December 2015, Vol. 25 No. 4, p. 181 - 188

Production of functional protein (including vaccine) using conventional technology in embryonated chicken eggs is laborious and lengthy. The use of chemical synthesis is not practical for peptides longer than 20 residues. In contrast, *in vitro* transcription and translation technology can directly utilize polymerase chain reaction (PCR) product as template for vaccine synthesis within two hours accurately. Moreover, up to 1-10 mg/ml protein can be produced using the technology compared to conventional method that only gives approximately one dose per egg. In this review, advantages and disadvantages of animal vaccine generation using conventional and *in vitro* methods would be described. *In vitro* transcription and translation technology can be considered as the most practical and efficient technique for rapid screening and generating new animal vaccines.

(Author)
Key words: Vaccine, transcription, translation, *in vitro*

UDC 636.082.4
Pamungkas FA (IRIAP, Bogor)
Wina E (IRIAP, Bogor)
Characteristics and Applications of Nanoparticles in Manipulation of Livestock Reproductive Hormones (Orig. Ind.)
Wartazoa December 2015, Vol. 25 No. 4, p. 171 - 180

The research on hormone packaged in very small size particles began to develop in recent years. Nanoparticles are defined as particulate dispersions or solid particles with a polymer used as a component of potential hormone carrier as effective drug because of their ability to circulate and to release in a controlled period in the body. This review describes a variety of methods, characteristics and applications of nanoparticles hormones usages for animals. In general, several studies indicated that the formation of the hormone nanoparticles using polymer accompanied by distributing a good and stable of molecular mass, can be used as a carrier component of hormones as well as considering the negative effect.

(Author)
Key words: Nanoparticles, hormones, characteristics

UDC 636.085.57:636.5
Nurfaizin (Maluku AIAT, Ambon)
Matitaputty PR (Maluku AIAT, Ambon)
Use of Carotenogenic *Neurospora* in Fermentation of Agricultural Byproduct for Poultry Feed (Orig. Ind.)
Wartazoa December 2015, Vol. 25 No. 4, p. 189 - 196

Agricultural byproduct is biomass that potential to partly subtitute the conventional feed. However, there are some constraints such as high fiber, low protein and carotene contents. One of the efforts to improve the nutritive value of agricultural byproduct is fermentation using carotenogenic *Neurospora*. This fungi easily and readily grows on substrate fermented in aerobic condition. *Neurospora* fermentation is able to reduce crude fiber, to increase crude protein and carotene content of substrate. Utilization of *Neurospora* fermented product as poultry feed ingredients increased productivity and product quality more efficiently.

(Author)
Key words: Feed, fermentation, carotene, *Neurospora*

UDC 636.084.52
Haryanto B (IRIAP, Bogor)
Technology in Feeding Management to Increase Ruminant Productivity (Orig. Eng.)
Wartazoa December 2015, Vol. 25 No. 4, p. 197-205

Traditional feeding management in ruminants is defined by allowing the animal to find their own feed, consisted of raw materials of grasses and leguminous foliages as much as possible (*ad libitum*) to get a high productivity; however, it needs longer period of time to reach maximum level of production. An advanced feeding management of ruminant is defined as: (1) Processing feed ingredients to improve the nutritive value; (2) Supplementing the animal with substances into the dietary formula to manipulate the rumen ecosystem either by reducing the protozoa population, increasing the concentration of certain nutrients, changing the rumen characteristics; and (3) Changing the site of digestion of the nutrients to increase the absorption and feed utilization. Many research works have been carried out to evaluate the effects of process technology on the efficiency of feed utilization. Improving feeding management will increase livestock production.

(Author)

Key words: Feeding management, supplement, rumen ecosystem, productivity

WARTAZOA Vol. 25 No. 4 Th. 2015 Hlm. 159-170 DOI: http://dx.doi.org/10.14334/wartazoa.v25i4.1226

Indonesian Dairy Industry Perspective within the ASEAN Economic Community

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(Accepted 27 January 2015 – Revised 6 July 2015 – Approved 23 October 2015)

ABSTRACT

Many of Indonesian main manufacturing industries based on agriculture should be more responsive to the challenging domestic and global strategic environment, including the newly emerging ASEAN economic community (AEC) which will be effectively implemented by the end of 2015. Dairy industry in Indonesia is a very potential business to play significant role in the ASEAN market based on the existing dairy population, feed resources and the number of dairy farmers. Recently, small and medium enterprise (SME) model in Java has been developed for processing milk. They provide higher farm gate prices under partnership agreement which created a good benchmark and business model for the future dairy industry to adopt. This new attractive business environment gives higher return to the farmers as opposed to the tradition of paying low farm gate milk prices. As feed represents 80% of the total production cost, special attention must be given to land availability to increase feed supply in terms of quality and quantity. Consequently, sustainable support from both central and local government is very critical to keep the partnership model between farmers and the SME milk processing. It also opens new opportunity to increase the linkage more closely between producers and milk processing plants.

Key words: SME dairy industry, Indonesia, ASEAN economic community

ABSTRAK

Perspektif Industri Susu di Indonesia dalam Masyarakat Ekonomi ASEAN

Industri-industri manufaktur utama Indonesia berbasis pertanian harus lebih responsif terhadap tantangan strategis baik domestik maupun global, termasuk masyarakat ekonomi ASEAN (MEA) yang akan efektif dilaksanakan pada akhir tahun 2015. Industri susu di Indonesia adalah bisnis yang sangat potensial untuk berperan signifikan di pasar ASEAN berdasarkan populasi sapi perah, sumber pakan dan jumlah peternak yang ada. Baru-baru ini, model usaha mikro, kecil dan menengah (UMKM) untuk pengolahan susu telah berkembang di Jawa. Usaha ini memberikan harga di tingkat petani lebih tinggi berdasarkan perjanjian kemitraan yang menciptakan tolok ukur yang baik dan dapat diadopsi sebagai model bisnis industri susu di masa depan. Lingkungan bisnis baru yang menarik ini memberikan pendapatan peternak yang lebih tinggi dibandingkan model tradisional yang menyebabkan peternak menerima harga susu lebih rendah. Kenyataan bahwa biaya pakan mencapai 80% dari total biaya produksi, maka perlu perhatian khusus pada ketersediaan lahan untuk meningkatkan pasokan pakan dari segi kualitas dan kuantitas. Sehubungan dengan itu, dukungan pemerintah pusat dan daerah yang berkelanjutan sangat penting untuk menjaga kesinambungan kemitraan antara peternak dan UMKM pengolahan susu. Hal ini membuka peluang baru untuk meningkatkan hubungan yang lebih erat antara produsen dan pabrik pengolahan susu.

Kata kunci: Industri susu UMKM, Indonesia, masyarakat ekonomi ASEAN

INTRODUCTION

Indonesia strong annual economic growth of 6% per year for the last three years 2010-2012, has been absolutely driven by the manufacturing and services sectors with some structural changes in production. However, agriculture sector remains the largest sector, in terms of employment since this sector has more than 50% of the total national workforce, while consistently plays significant role in export earning, that is from around 12% of merchandise exports or about 30% of non-oil and non-gas exports (BPS 2014). Since the agricultural sector is mainly based on domestic resources, therefore it is a slightly affected by global economic fluctuations. Meanwhile, as a natural resource based sector, Indonesian agriculture somewhat is insulated from domestic macroeconomic shocks such as monetary, exchange rate and fiscal fluctuations. Many of Indonesian main manufacturing industries are agricultural based and if it is to be a basis for the recovery and growth of the economy, Indonesian agriculture must, therefore, become more responsive to

the challenging domestic and global strategic environment, including the newly emerging ASEAN economic community (AEC) which will be effectively implemented by the end of 2015.

In Indonesia, milk and milk products have long been considered as strategic commodities to meet nutritional food requirement for human being, therefore, it should be reliable for value added taxes free to keep it growing and becoming more competitive, especially in the marketing and processing industry. Dairy products will continue to provide opportunities for the milk processing industry to expand its capacity since more than 45 million people of the consuming class, that is around 53% of the population in cities producing 74% of gross domestic product (GDP), 55 million skilled workers in the Indonesian economy and $ 0.5 trillion market opportunity in consumer services, agriculture and fisheries, resources and education (Oberman et al. 2012). Dairy sector in Indonesia, with its prevalence capacity to produce domestic fresh milk year round, coupled with growing middle and upper income groups as potential consumers, has been experiencing substantial high raw material import volume (78%) for the milk processing industry. Along with Indonesia stable political climate, increasing per capita milk consumption and a growing awareness toward general health status of the society as a direct benefit from dairy product, the Indonesian milk processing industry will continue to grow and provide opportunities for Indonesian economy. This perspective is very visible from the fact that the dairy manufacturers are expanding their capacity, to stimulate domestic dairy industry which is currently growing at an annual rate of 8% (Darmawan 2013).

Although several major dairy farms are expanding their herd number, the overall growth of domestic fresh milk production remain limited to some degree and tends to put in a relatively stagnant position of domestic milk production, which in turns reduces its contribution to meet the demand from growing dairy industry in the near future. This is a true challenge to the sector, especially when the industry is building its capacity towards implementable AEC in 2015. Indonesian dairy milk industry has been supplied fresh milk from dairy farmers, where majority involved as member of a local dairy cooperative union (KUD). The KUD then collects and sends the milk to milk processors. Tawaf et al. (2009) stated that only 8% of milk from individual dairy farmers goes directly to final consumers as fresh liquid milk or yoghurt drinks. Milk processors may manufacture this milk into liquid milk, condensed milk, powdered, ice cream and yoghurt. This will follow by packaging and labeling of milk product and picked up by distributors before selling into supermarkets or outlets where final consumers need to buy it. This paper reviews some of potential competitiveness of Indonesian dairy industry to take advantages of higher production value within the country as well as in the region within the frame of AEC in 2015.

IMPLICATIONS OF AEC ON INDONESIA DAIRY INDUSTRY

During the 9th Summit of the Association of Southeast Asian Nations (ASEAN) in Bali, Indonesia in 2003, the ASEAN Heads of Government passed the Declaration known as the Bali Concord II which pronounced the establishment of an ASEAN Community by the year 2020. The declaration determined to achieve higher levels of economic dynamism, sustained prosperity, inclusive growth and integrated development of ASEAN as well as in order to promote greater political, security and economic cooperation in the region. One of the frameworks in this accord was the ASEAN economic community (AEC) which envisions economic integration in the region through the concept of free flow of goods, services, investments, capital and skilled labor (AEC 2008). In 2007, during the 12th ASEAN Summit in Cebu, Philippines, the ASEAN Leaders agreed to accelerate the establishment of the ASEAN Community by 2015, as envisioned in the ASEAN Vision 2020 and the ASEAN Concord II and signed the Cebu Declaration on the Acceleration of the Establishment of an ASEAN Community. In the following Summit in Singapore in November 2007, the Leaders signed and adopted the AEC blueprint which serves as the detailed master plan in establishing the AEC by 2015. The AEC blueprint stated that it "will transform ASEAN into a single market and production base, a highly competitive economic region, a region of equitable economic development, and a region fully integrated into the global economy" (AEC 2008).

The AEC goal of regional economic integration rests on four pillars, namely (1) Single market and production base; (2) Competitive economic region; (3) Equitable economic development; and (4) Full integration into the global economy. Under each pillar are core elements which require specific actions and strategic approaches to realize the intended outcomes (Table 1). These characteristic are inter-related and mutually reinforcing, in addition incorporating the required elements of each characteristics shall ensure the consistency and coherence of these elements as well as their implementation and proper coordination among relevant stakeholders.

The food, agriculture and forestry sectors are important component under the single market and production base pillar of the AEC. Specifically, the

AEC intents to implement further enhancement of intra and extra-ASEAN agricultural trade through the reduction and removal of tariffs via the CEPT-AFTA, removal of non-tariff barriers and the adoption and harmonization of quality management systems for food safety. This will also include cooperation and technology transfer with international organizations and private sector as well as market access through ASEAN agricultural cooperatives. These aspects would be very much relevant with the business of dairy sector in Indonesia. Under ASEAN trade in goods and agreement (ATIGA), the ASEAN six countries, including Indonesia, currently impose zero tariffs on all dairy products based on ASEAN harmonized tariff nomenclature (AHTN) in 2012. Cambodia, Lao PDR, Myanmar and Vietnam impose non-zero tariffs in some dairy products in the AEC period (Table 2). The most favorable nation (MFN) tariff rates for agricultural products remain low in most of the ASEAN countries. The reduction and or elimination tariffs would increase the value of intra and extra-ASEAN trade in the agricultural, including dairy sector.

Given the steady decline in tariffs for dairy products in the region, with around 600 million populations where 42% lives in Indonesia, important trade aspect would be the most challenging issue for dairy industry in Indonesia. This will also be driven by positive economic indicator in 2014 as was considered fairly good in terms of its GDP annual growth rate measure of 5.1% along with its moderate inflation rate

Table 1. Pillars and core elements of the ASEAN economic community blueprint

Core elements	Key strategic areas of action
Pillar 1: Single market and production base	
A1. Free flow of goods	Removal/reduction of tariffs
	Removal of non-tariff barriers
	Rules of origin
	Trade facilitation
	Customs integration
	ASEAN single windows
	Standards and technical barriers to trade
A2. Free flow of services	Remove substantially all restrictions on trade and services under ASEAN framework agreement on services
	Scheduled commitments for national treatment limitations
	Complete mutual recognition arrangements (MRAs)
	Liberalization measures of the financial services sector
A3. Free flow of investment	ASEAN investment cooperation is being implemented through ASEAN investment agreement (AIA)
	Provide enhance investment protection as well as facilitation and cooperation likewise promotion and awareness
A4. Free flow of capital	Strengthening ASEAN capital market development and integration
	Allowing greater capital mobility
A5. Free flow of skilled labour	Manage mobility or facilitate entry for the movement of natural persons engaged in trade in goods, services and investments
A6. Priority integration sectors[*)]	Twelve priority integration sectors develop a roadmap which combines specific initiatives and the broad one
	Allows the region to focus its limited resources and deep integration in these critical areas while provides ASEAN members to jointly develop a stronger sense to economic integration prior to a broader roll-out
A7. Food, agriculture and forestry[*)]	Monitor implementation of common effective preferential tariffs for ASEAN free trade area (CEPT-AFTA) within agriculture and forestry products
	Harmonization and application of quality standards for food safety
	Cooperation and technology transfer with international organisations and private sector
	Market access through ASEAN agricultural cooperatives

[*)]including the two important components on the first pillar

Table 1. Pillars and core elements of the ASEAN economic community blueprint (continued)

Core elements	Key strategic areas of action
Pillar 2: Highly competitive economic region	
B1. Competition policy	Establish a network of authorities responsible and develop regional guideline
	Encourage capacity building and adoption of best practices
B2. Consumer protection	Establish ASEAN coordinating committee on consumer protection (ACCCP)
B3. Intellectual property rights	Fully implemented Intellectual Property Rights action plan
B4. Infrastructure development	Transport cooperation
	ASEAN framework agreement on multi-moda transport and transport facilitation: Land, maritime and air transport
	Develop information infrastructure and energy cooperation
B5. Taxation	Build bilateral agreements on avoidance of double taxation
B6. E-commerce	Develop E-commerce best practices of regulations
Pillar 3: Equitable economic development	
C1. SME development	Timely implementation of ASEAN policy blueprint for SME development
C2. Initiatives for ASEAN integration (IAI)	Give the direction and sharpen the focus of collective efforts to narrow the development gap not only between ASEAN but between ASEAN and other countries as well
Pillar 4: Full integration into the global economy	
D1. Coherent approach towards external economic relations	Establish a system for enhanced coordination for free trade and comprehensive economic partnership
D2. Enhanced participation in global supply networks	Continuing the adoption of international best practices and standards in production and distribution
	Developing a comprehensive package of technical assistance to upgrade capability and productivity

Source: AEC (2008)

Table 2. Tariff implementation of dairy products in ASEAN countries

Countries	Average MFN applied (%)*)	ATIGA tariff by year (%)**)			
		2012	2013	2014	2015
Brunei Darussalam	n.a	n.a	n.a	n.a	0
Cambodia	15.2	5	5	5	0-5
Indonesia	7.5	0	0	0	0
Lao-PDR	na	5	5	5	5
Malaysia	8.9	0	0	0	0
Myanmar	8.6	3-5	3-5	3-5	0-5
Philippines	9.9	3-7	0	0	0
Singapore	1.4	0	0	0	0
Thailand	29.9	0	0	0	0
Vietnam	16.2	5	5	5	0-5

n.a: Not available; MFN: Most Favorable Nation; ATIGA: ASEAN Trade in Goods and Agreement

Source: *)WTO (2014); **)ASEAN Tariff Schedules 2013

of 6.23% (BPS 2014). Domestic consumption is the major driver of Indonesia economic activity, where consumption accounted for 61% of national GDP and is expected to reach 65% in the next 20 years. This would be a tremendous potential for Indonesia consumer market as the economy continues to grow and the consumers become wealthier with obvious tend to consume preferably non-staple food, such as dairy products. Annual consumer spending is projected to increase by 7.7%, while current trends indicate that

Indonesia is likely to become the seventh-largest economy in the world by 2030 compared with current position as the sixteenth (Oberman et al. 2012). It is very obvious that Indonesian economy is transforming rapidly with growing young population while quickly urbanizing, hence empowering the growth in income. This growth of consuming class would indicate prospective future to worldwide business and investors for its considerable new opportunities.

The promoting of emerging creative small and medium enterprises will be fully supported by the new government, which is actively pursuing smallholder farmers to gain better life. This objective is indeed in line with the priority initiative as indicated in the AEC Blueprint particularly to enhance its pillar number C, i.e. the strategic importance of small and medium sized enterprises for equitable growth. The development of facilitating access to finance, information, market and technology are very important since most of the dairy industry is characterized by smallholders with limited size of cow ownership and marketing of their fresh milks is only one option through cooperatives. Combined with Indonesia stable political climate, stronger per capita milk consumption, and greater awareness of the health benefits from dairy products, dairy industry will continue to provide opportunities for the economy to grow. Indonesian economy's growth could also benefit from current powerful trends to meet the challenges ahead through improvement of transforming consumer services, boost productivity in agriculture and fishery, creating a smart economy resource as well as skill building investment.

In addition to those factors above, Indonesia has improved its position by four up stepping in the world economic forum's global competitiveness index in 2014-2015, which places Indonesia currently at 34th place out of 144 countries, compared to previous position on 38th place (2013-2014) (WEF 2014). The global competitive index measures the institution, policies, as well as factors that set sustainable current and medium period levels of economic prosperity among 144 countries around the world, where Indonesia improved steadily from rank 50th since the year 2012-2013. The successful increase in rank is due mainly to improvements in macroeconomic environment experiences that significantly increase recently. This increase reflects continuous improvements in Indonesian national economic performance as a positive indicator to enhance competitiveness within the ASEAN region. Nevertheless, it also shows that Indonesia still lags behind from some regional countries such as Malaysia and Thailand with their current ranks of 20th and 31th place, respectively. A serious effort and commitment need to be implemented to sort out some of critical points such as the needs to develop infrastructure continually, bureaucratic reform for efficient services and corruption eradication. Table 3 shows a more detail of the global competitiveness index ranking in 2014-2015 and in 2013-2014 of the ASEAN countries.

POTENTIAL GROWTH OF INDONESIA DAIRY PRODUCTION

It has been well known that the role of the small holder producers in the dairy industry is very significant. They produced about 90% of the total fresh milk production in this country. Milk production increased rapidly by 18.7% during the period of 2009-2013, or 4.7% annually (DGLAHS 2013) and that daily fresh domestic milk production has reached 1.02 million ton. The most recent census on cattle and buffalo population indicated that dairy cattle population was close to 622 thousand heads and 59.95% of it were cows (DGLAHS 2013). East Java is considered the

Table 3. The global competitiveness index (GCI) 2014-2015 and 2013-2014 rankings of ASEAN countries

Countries	GCI 2014-2015 rank (out of 144)	Score (1-7)	GCI 2013-2014 rank (out of 148)
Malaysia	20	5.16	24
Thailand	31	4.66	37
Indonesia	34	4.57	38
Philippines	52	4.40	59
Vietnam	68	4.23	70
Lao-PDR	93	3.91	81
Cambodia	95	3.89	88
Myanmar	134	3.24	139
Brunei Darussalam	n.a	n.a	n.a
Singapore	n.a	n.a	n.a

n.a: Not available

Source: WEF (2014)

most important milk production area of Indonesia which shares 50% population and 56% share of the total milk production of this country (Table 4). Seven major milk producing districts in the country include Malang and Pasuruan in East Java, Semarang and Boyolali in Central Java, Lembang and Bandung in West Java along with Sleman in Yogyakarta. The average yield is between 10-12 liters/cow/day. This dairy farming has been involving 125 thousand farmers which dispersed into 1,500 farmer groups within 95 primary cooperatives. It is predicted that there is a potential calf's birth of 140 thousand heads or approximately require 70 thousand heifers as replacement stocks per year. Table 4 explains a more detail dairy population and its production from the main provinces that serve as the milk production center areas in Indonesia.

In addition to the current production areas on Java, the government has supported the initiative for developing dairy industry in new areas out of the Java Island. The new areas include Sumatra (North Sumatra, Jambi, West Sumatra and South Sumatra), South Kalimantan and South Sulawesi. The support on this initiative was mainly based on the fact that there is considerable and potential feed resources in this region to solve basic problem that feed requirements would be no longer persistence due to land shortages such as in the island of Java. In addition, more agricultural and or agro-industry by products are available abundantly as extensive estate plantations are mostly located in these areas. Since the availability and efficient use of the feed resources are the primary drivers of performance to maximize dairy productivity, therefore, the areas will have comparative advantage in terms of geographical proximity as they are located close to the neighboring countries such as Malaysia, Singapore and Thailand. This is also relevant to previous study by Devendra & Leng (2011) that reported feed security is fundamental to the management, extent of use, conservation and intensification for livestock productivity enhancement.

The national per capita milk consumption currently stands at 11.7 l/year, which is obviously lower compared to that in some ASEAN countries such as Thailand (30.1 l), Malaysia (30.0 l) and Vietnam (15.0 l) (Darmawan 2013). It is expected that national milk consumption would increase by 8% this year compared to that of 2012. This expectation of increase would be triggered by increased in human population (1.45% per year), consumer's income, and improved nutrition awareness. In recent years, consumers prefer to drink more liquid ready fresh milk and UHT milk which continue to dominate the market.

Current local milk production has contributed only 22% of the total milk supply, which justify that Indonesia has to import the rest of 78% to fulfill the demand equals to 178.8 thousand ton of milk and milk products or equivalent to USD 603 million in 2012 (DGLAHS 2013). The tremendous potential milk production and market for the country along with its initiative to extend the production areas would also stimulate the dairy industry to take advantage of export markets within the perspective of new markets of the ASEAN region.

Milk quality as one of important aspects in the effort to improve the industry's competitiveness in the region is among high priorities for the government to facilitate the industry to rise standards of its products. Quality of fresh milk is generally measured by the bacteria content in terms of total plate count (TPC), which range from 500 thousand to 1 million. Price incentives have been used to encourage better farm management practices and higher quality milk, however some are still perform far below the minimum standard of national quality standard (SNI), i.e. a maximum of 1 million for TPC, a minimum of 11% for total solids, and milk content (protein of minimum 2.7%; fat minimum of 3% and solid non fat at minimum of 8%). Dairy cooperative union (KUD) collects and measures bacteria content of the fresh milk from farmers to determine the quality and price paid to farmers. Despite improving technical aspect to increase milk quality under farmer's condition, it is also important to seek other approaches in cope with the role of the KUD. Devitt et al. (2013) stated that application of collective action in the organization of milk cooperatives need to be implemented, this include extension program involving advisors and dairy farmers along with building industry's capacity.

Table 4. Dairy cow's population and milk production by main provinces, 2013

Province	Dairy cow population (head)	Dairy cow population (%)	Milk production (ton)	Milk production (%)
East Java	309,775	49.8	570,082	56.0
Central Java	152,220	24.5	106,224	10.4
Yogyakarta	3,613	0.6	3,260	0.3
West Java	147,958	23.8	326,115	32.0
Others	8,414	1.4	12,249	1.2
Total	621,980	100.0	1,017,930	100.0

Source: DGLAHS (2013)

Facilitated communication process and creating incentives are important to encourage agreement among different stakeholders.

TRADE OF FRESH MILK PRODUCT IN INDONESIA

Over 90% of daily fresh milk production from the smallholder farmers is collected by the KUD and supplied to the milk processing industry as major buyers. The industries produce milk powder, sweetened condensed milk, pasteurized milk, cheese, butter and others. Three types of consumer products dominate the market are powdered milk, sweetened condensed milk (SCM) and ultra high temperature (UHT) milk with their market share of 39, 35 and 26%, respectively (GAIN 2013). Consumers' preference for fresh products is expected to continue boosting fluid milk growth that has currently expanded to 17% annually over the past seven years. There are nine major milk processing companies in this country during 2011 in which Nestle is the biggest share to total market (47%). The Ministry for Industry reported that in 2011 milk powder was the product which dominated national consumption (43.3%), followed by sweetened condensed milk (20.4%). The use of milk as raw material for biscuits, ice cream, candy, chocolate and others are also high (27.5%). On the other hand, liquid milk consumption is considered low (8.5%) which consist of UHT (4.6%), sterilized milk (2.7%) and pasteurized milk (1.2%). This figure indicated that there is a huge potential to increase liquid milk production to meet the demand for consumption. To attract more participation in milk consumption, packaging may become another important issue despite it adds the production cost.

Indonesia imports various milk and cream, not concentrated nor sweetened or concentrated whether or not sweetened by various fat content by weight, not exceeding 1%, exceeding 1% but not exceeding 6%, that experienced slow in 2013 due to tight international supplies. During the period of 2008-2012 value of the milk and milk products imported was around USD 995.469 million shown an increasing of 5.3% per year (Table 5) (Kemenperin 2013). In the year of 2008-2009, it can be shown that all of the milk products had decreased significantly, especially for milk and cream, concentrated whether or not sweetened, in powder, granules or other solid forms, of a fat content, by weight not and exceeding 1.5%. This could be caused by impact of global financial crisis that started in mid 2007 and ended by end of 2008 to make shortages of milk supplied. Besides, depreciation of IDR exchange rate into US dollars may still influence the decreased. During the period of 2010-2012 most of milk products has increased steadily that indicated dairy industry has suffered from the global financial crisis. The main products imported were skim milk and whole milk products, where the main exporters came from European Union, New Zealand, USA and Australia.

The national union of dairy cooperative (GKSI 2013), a secondary cooperative plays the role in coordination primary milk cooperatives. One of GKSI responsibility is to negotiate milk price with major buyers. However, it always the case that low milk prices paid by the major milk processors, while low bargaining power at the GKSI side are obvious implication from the fact that more than 90% of the milk goes into this industry. Consequently, the ratio of farm gate milk price has decreased persistently from 0.53 to 0.62 during the period of 1999-2008 (Priyanti & Saptati 2009) or equivalent to 0.52 in 2013 calculated using current price (Table 6). This figure indicates that the price of fresh raw milk from farmers is less valued compared to that of imported full cream milk powder include 5% tax and 2.5% transport cost. The depreciation of rupiah to US dollar as shown by decreasing exchange rate in 2013 has also influenced the ratio of farm gate price to import. Therefore, one of the main crucial issues that need special attention from government point of view is to facilitate and to establish a control mechanism to improve milk price from the buyers. This step may stimulate farmer producers to naturally improve production performance at the attractive farm gate price.

One of the important measures in order to get better farm gate price is income over feed cost (IOFC), which take into consideration the change in the margin over feed cost. This will also allow to describe changes of milk to feed price ratio to estimate profitability when the price changes are largely driven by the price in time series. Wolf (2010) has indicated that IOFC is a better proxy to estimate dairy farm profitability, due to feed price volatility changes in the series of time period without any sign of a decline in milk price volatility. This case is most likely happened in Indonesia, smallholder dairy farm, where feed price increases year by year, while no significant increases on farm gate milk price. IOFC is fairly easy to calculate with readily available price data on feed efficiency and milk production.

COMPETITIVENESS OF SMALL-MEDIUM ENTERPRISE FOR MILK INDUSTRY

Issues on production efficiency and cost will eventually become the major factors and their consequences for the competitiveness of Indonesia milk industry within the perspective of the AEC 2015 market. In this instance, the small and medium enterprise (SME) will play key important role in national economy. The reasons are: (1) It covers 55.2

Table 5. Imported milk and milk products based on HS number, 2007-2012

Products	2008		2009		2010		2011		2012	
	Vol. (000 tonnes)	Value (000 USD)	Vol. (000 tonnes)	Value (000 USD)	Vol. (000 tonnes)	Value (000 USD)	Vol. (000 tonnes)	Value (000 USD)	Vol. (000 tonnes)	Value (000 USD)
Milk and cream (HS 40110)	345.6	3,362	563.6	5,889	496.8	4,536	852.1	7,518	894.6	8,433
Milk and cream (HS 40120)	1,856.3	18,057	814.2	8,509	76.5	699	99.8	880	39.7	374
Milk and cream (HS 40130)	323.4	3,145	161.3	1,686	724.1	6,611	1,077.6	9,507	1,454.0	13,707
Milk and cream (HS 40210)	30,107.5	292,855	22,806.7	238,330	44,376.0	405,153	50,120.9	442,216	51,811.6	488,428
Milk and cream (HS 40221)	14.3	138,471	6.7	70,229	12.2	111,458	21.5	189,841	22.3	210,458
Milk and cream (HS 40229)	20,930.0	203,586	7,962.3	83,206	11,133.0	101,647	15,578.3	137,447	12,719.7	119,909
Milk and cream (HS 40291)	199.7	1,943	13.3	139	13.6	124	6.9	60.5	2.2	21
Milk and cream (HS 40299)	371.2	3,610	346.8	3,624	969.7	8,854	1,012.8	8,936	1,226.6	11,563
Buttermilk (HS 40390)	2,528.7	24,597	1,674.4	17,498	3,411.6	31,148	2,462.2	21,724	2,052.6	19,349
Natural milk constituents (HS 40490)	134.9	1,312	90.4	944	524.8	4,791	166.4	1,468	155.0	1,461
Malt extract (HS 190190)	4,434.9	43,138	3.310.7	34,597	6,294	57,465	7,830.6	69,089	9,287.2	87,550
Nonalcoholic beverages (HS 220290)	5,432.9	52,846	3,782.9	39,532	4,782.8	43,667	4,055.7	35,783	3,629.6	34,216

Source: Kemenperin (2013); data has been analysed

Table 6. The dynamic of farm gate fresh milk price, imported full cream milk powder and their price ratio, 1999-2013

Year	Price FCMP (Rp/kg)	Price FCMP + 5% tax (Rp/kg)	Price FCMP + 5% tax + transport cost (Rp/kg)	Price equivalent to fresh milk (Rp/l)	Farm gate milk price (Rp/l)	Ratio farm gate milk price to import
1999	14,055	14,758	15,053	1,882	1,000	0.53
2000	17,020	17,871	18,228	2,279	1,137	0.50
2001	17,921	18,871	19,194	2,399	1,411	0.59
2002	12,882	13,526	13,796	1,725	1,562	0.91
2003	15,976	16,774	17,110	2,139	1,612	0.75
2004	19,927	20,923	21,342	2,668	1,647	0.62
2005	20,855	21,898	22,355	2,792	1,756	0.63
2006	21,779	22,868	23,326	2,916	1,988	0.68
2007	43,055	45,208	46,112	5,764	2,431	0.42
2008	38,815	40,756	41,751	5,196	3,200	0.62
2009	37,954	39,852	40,848	5,106	3,200	0.63
2010	34,630	36,362	37,271	4,659	3,300	0.71
2011	33,143	34,800	35,670	4,459	3,400	0.76
2012	36,818	38,659	39,625	4,953	3,600	0.73
2013	54,285	56,999	58,424	7,303	3,800	0.52

FCMP: Full cream milk products

Source: Priyanti & Saptati (2009) and further analyzed data

million business unit approximately 99% of total business; (2) Labor and work force opportunity for 101.7 million people (97,24% workers); (3) Gross domestic product contribution of 57.1% (IDR 3.4 trillion); and (4) Investment value for IDR 927 trillion (44.89%) in 2012 (Ministry of Small-Medium Enterprise and Cooperatives 2012).

One of the indicator for measuring competitiveness is domestic resource cost ratio (DRCR) approach, which recent study by Sirajuddin et al. (2013) indicated that dairy farming in Enrekang and Sinjay, South Sulawesi were incompetitive with the value of 1.297 and 2.059, respectively. A business with 0< DRCR <1 categorized as having good competitiveness, on the other hand, with DRCR >1 is economically inefficient or do not have any competitiveness. This is due to the higher domestic resource cost at its social price compared to that of additional output tradable value (Masters & Winter-Nelson 1995). This implies that this business does not have any comparative advantage that considered as incompetitive business. Fortunately previous studies by Rachman (1998) had shown that dairy farming in Bogor and Bandung, West Java had various DRCR in a range of 0.63-0.86. This could be due to better economies scale of dairy farming in West Java with more processing industry involved. This research has been done on three groups of credit scheme, i.e. small scale dairy farming (<4 heads/farmer), recommended credit scheme of 7-10 and >13 heads/farmer in milk industry partnership approach. Furthermore, dairy farming in East Java has shown that farm gate price influenced by several components, i.e. milk price base, competitive incentives, loyalty, transport cost and feed incentives. These factors may build fresh milk price structure that has been bought by processing industry (Nugroho 2012). Feed and fuel price are indicators that may fluctuate dynamically and affected to overhead production cost at farmers' level.

In coping with the SME for processing milk industry, one of the milk businesses in Bogor, West Java, PT. Cisarua Mountain Dairy (Cimory Indonesia) implements the company strategy under their partnership through providing the highest farm gate prices to its dairy farmers. The strategy is being considered and provides a good benchmark and business model for the dairy industry to adopt. On the average, paid milk price is 10% higher using TPC and TS as the main criteria. To minimize bacteria contents during transportation fresh milk is delivered directly to the processing factory. This is an opportunity to increase the value along milk supply chain by linking the processing industry more closely to producers.

The company sells premium milk and dairy products such as fresh milk, yoghurt, pasteurized milk, UHT and cheese. These products require good quality

of fresh milk from farmer's producer as a reward from getting attractive farm gate price. A price incentive is used to encourage better farm management practices as it is required by higher quality milk. It is reasonable that the industry provides technical services to the farmers as an instrument to achieve higher quality milk through direct participation and observation at all points of the process line. It took quite a long time for the farmers, however, to adapt to this mechanism at the very beginning, but now farmers get used to it as a standard daily management practices. Consequently, in 2006, they were capable of producing milk at 2.5 tons/day and in 2012; it became 15-20 tons/day involving 200 farmers and approximately 1,000 cows (Cimory Indonesia 2012).

Furthermore, the company has also launched a new processing factory in the District of Semarang, Central Java, in 2012 as a replication of a partnership model developed earlier in the District of Bogor, West Java. This could be due to good prospect of dairy business as shown by the previous factory, so that having a trend toward enlarging plant factory and increasing intensification in terms of output per factory on the entire system. The economic efficiency of dairy business is the main goal of the company, with one of the success factors is to build a mutual good partnership with farmers by implement good payment for good milk quality. Atzori et al. (2013) has reported that the main four components were very crucial to get contribution to profitability that associated with managerial and technical aspects, those are herd profile, milk quality and payment, good management and cow's reproduction rate. Reproduction would be the highest effect on economic importance of profitability, since this is true that it affected to milk production and in turn accounted for most of the economic benefits in programs with superior economic performance.

Strategy to group farmers into KUD has been ideally built independently on the basis of on-farm activities and the corporation management is proofing a success story by the SME milk processing which focuses at the marketing efficiency aspects. This strategy could be used extensively by similar companies through optimizing production partnership approach between SME and large milk processing. Moreover, contract of manufacturing which creates some benefits from guaranteed investment, production capacity, efficient promotion cost and marketing activities. The SME milk industry has to further be supported consistently by the government in order to achieve their goals gradually. The supports include: (1) Training and implementing food safety principles; (2) Applying method to produce good food; (3) Implementing food safety management based on HACCP principles; and (4) Certifying international quality assurance (ISO 9000, 22000, SQF). In addition, principles on good farming practices, good handling practices, good distribution practices, good retailing practices and good consumption practices need to be applied as the whole systems to present good quality food from farm to table. This needs to strengthen partnership cooperation between smallholders farmers and medium enterprises to produce fresh milk basis on a market driven by government program, such as school milk program.

In case of Indonesia, where most of the fresh milk product goes into processing factory, farm gate price would be the most important factor that can drive in achieving good milk production. There should be a mutually price benefit between the two parties, when there is a price incentive for farmers, dairy farming would be attractive to them and they may produce good quality milk. This will also need to have effective and integrated institutional marketing aspect from smallholder farmers as producers and medium scale of processing enterprise as consumers. Other than farm value cost is considered important that need to be discussed such as packaging, labeling, etc.

Integrated approach among institutions involved: cooperatives, livestock production and animal health services, bank and financial institution, private sectors that help farmers for some aspects: technical service, training, provision on information, etc. Cooperatives leaders need to build the cooperatives capacity to provide collective services required to attain high quality milk production-that is cooperatives are agents of change. Cooperative reform is then the first and highest priority to expand the dairy business, which they may need technical advice on how to gradually improve quality standards and what improvement measures to take. They need capacity building for extension workers who will disseminate information, training to farmers and monitor their practices as well as for the cooperatives staff working at milk collection centers.

CONCLUDING REMARKS

Indonesia has a potential opportunity to improve milk industry towards AEC market due to its 2nd place of potential dairy population after Thailand. Domestic milk production needs to be increased beyond current capacity that indicates a strong challenge and high opportunity to improve domestic dairy production.

Building a partnership model between farmers and the SME milk processing plants, which are capable of paying higher farm gate prices to dairy farmers, should not become only 'a good concept on a paper' but it has to be an effort to 'put a theory into practice' since it is an opportunity to increase the milk value along the

supply chain. Good marketing development and strategy will further prove that expansion of the SME milk industry partnership model could promote mutual equitable benefit.

In reference to all the above thought and practices, the Government of Indonesia is developing a comprehensive blueprint for the development of dairy industry and its follow up action plans to face the ASEAN economic community starting in 2015. All dairy stakeholders are expected to have their commitments to achieve the targets, among others. This will include: (1) application of good farm management practices; (2) provision of attractive farm gate prices to guarantee a feasible dairy farming; (3) building a partnership structure that mutually benefit between farmers and the SME; and (4) increasing the role of central and local government to support this industry.

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WARTAZOA Vol. 25 No. 4 Th. 2015 Hlm. 171-180 DOI: http://dx.doi.org/10.14334/wartazoa.v25i4.1227

Karakteristik dan Aplikasi Partikel Nano dalam Manipulasi Hormon Reproduksi pada Ternak

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(Diterima 10 Juli 2015 – Direvisi 13 Oktober 2015 – Disetujui 23 Oktober 2015)

ABSTRAK

Penelitian hormon yang dikemas dalam partikel nano mulai berkembang dalam beberapa tahun terakhir ini. Partikel nano sebagai partikel dispersi atau padat berukuran sangat kecil yang dilapisi dengan polimer dapat digunakan sebagai komponen pembawa hormon yang potensial karena kemampuannya dalam meningkatkan stabilitas hormon dan melepaskan hormon secara terkontrol dalam waktu lama di dalam tubuh ternak. Ulasan ini menjelaskan berbagai metode, karakteristik dan aplikasi partikel nano dalam pemanfaatan hormon pada hewan. Secara umum, beberapa hasil penelitian menunjukkan bahwa pembentukan hormon partikel nano menggunakan polimer dengan distribusi massa molekul yang baik dan stabil dapat digunakan sebagai komponen pembawa hormon, dengan tetap mempertimbangkan adanya efek negatif.

Kata kunci: Partikel nano, hormon, karakteristik

ABSTRACT

Characteristics and Applications of Nanoparticles in Manipulation of Livestock Reproductive Hormones

The research on hormone packaged in very small size particles began to develop in recent years. Nanoparticles are defined as particulate dispersions or solid particles with a polymer used as a component of potential hormone carrier as effective drug because of their ability to circulate and to release in a controlled period in the body. This review describes a variety of methods, characteristics and applications of nanoparticles hormones usages for animals. In general, several studies indicated that the formation of the hormone nanoparticles using polymer accompanied by distributing a good and stable of molecular mass, can be used as a carrier component of hormones as well as considering the negative effect.

Key words: Nanoparticles, hormones, characteristics

PENDAHULUAN

Kontrol biologis dalam budidaya di bidang peternakan dapat dilakukan melalui manipulasi lingkungan, hormonal dan genetik. Kontrol hormonal pada proses reproduksi, ekskresi hormon gonadotropin (GTH) seperti *follicle stimulating hormone* (FSH) dan *luteinizing hormone* (LH), dikendalikan oleh *gonadotropin releasing hormone* (GnRH) yang merupakan hormon peptida. Hormon tersebut memiliki daya kerja yang pendek dalam darah karena cepat terdegradasi oleh endopeptida dan eksopeptida yang terdapat pada hipofisis, ginjal dan hati. Salah satu cara mempertahankannya adalah dengan penyuntikan hormon berulang, walaupun dapat menyebabkan stres pada ternak (Zohar & Mylonas 2001). Metode aplikasi hormonal dalam bentuk partikel nano dapat digunakan sebagai kontrol biologis melalui perlindungan hormon dari degradasi cepat selama transportasi sebelum mencapai organ target dan pengendalian pelepasan agen bioaktif secara berkelanjutan (Rather et al. 2013).

Partikel nano merupakan suatu partikel dispersi atau padat dengan ukuran antara 10-1000 nm (Mohanraj & Chen 2006). Penyatuan hormon pada matriks partikel nano tergantung pada metode pembuatannya. Polimer partikel nano yang dilapisi hidrofilik polimer, potensial digunakan sebagai komponen pembawa hormon karena mampu meningkatkan kinerja hormon melalui perlindungan stabilitas, serta *life time* pelepasan secara terkontrol di dalam tubuh ternak (Bhadra et al. 2002; Vila et al. 2002; Kommareddy et al. 2005).

Tujuan utama dalam merancang partikel nano sebagai pembawa hormon adalah untuk mengontrol ukuran partikel, mempertahankan sifat permukaan dan melepaskan agen bioaktif secara spesifik dengan dosis optimal. Keuntungan menggunakan partikel nano sebagai pembawa hormon adalah: (1) Ukuran dan permukaan karakteristik partikel nano dapat dimanipulasi sesuai keinginan; (2) Kontrol jumlah pelepasan hormon dapat diatur sesuai target; (3) Perlindungan degradasi partikel dapat diberikan; dan

(4) Penggunaan hormon dapat melalui mulut, hidung, parenteral, intraokular dan sebagainya. Disamping beberapa keunggulannya, partikel nano juga memiliki keterbatasan diantaranya dengan ukuran yang kecil dan luas permukaan yang besar dapat menyebabkan penggabungan partikel-partikel sehingga membuat penanganan fisik partikel nano menjadi sulit, baik dalam bentuk cair maupun kering (Mohanraj & Chen 2006).

METODE PEMBUATAN PARTIKEL NANO UNTUK HORMON

Partikel nano dapat dibuat dari berbagai bahan utama seperti protein, polisakarida dan polimer sintetis. Pemilihan komponen utama tergantung beberapa faktor diantaranya ukuran partikel nano, sifat hormon, karakteristik permukaan, tingkat biodegradasi, biokompatibilitas, toksisitas, kontrol pelepasan hormon dan antigenisitas produk akhir (Kreuter 1994). Pembuatan partikel nano umumnya menggunakan empat metode yaitu: (1) Polimer terdispersi; (2) Polimerisasi monomer; (3) Gelasi ionik atau koaservasi polimer hidrofilik; dan (4) Fluida/pelarut super kritis (Mohanraj & Chen 2006).

Metode polimer terdispersi

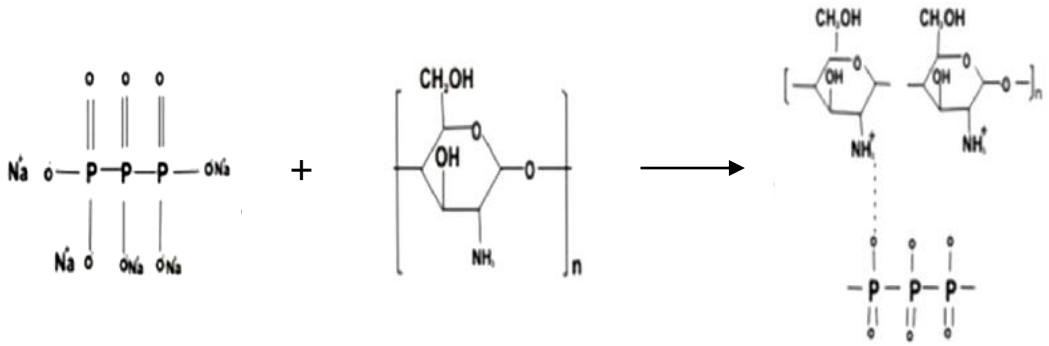
Metode umum yang digunakan untuk menyiapkan partikel nano yang ramah lingkungan antara lain dari *polylactic acid* (PLA), *polyD L-glycolide* (PLG), *polyD L-lactide-co-glycolide* (PLGA) dan *polyalcylcyanoacrylate* (PACA) (Kompella et al. 2001; Li et al. 2001; Ravi et al. 2004). Metode ini dapat digunakan dalam berbagai cara yaitu: (1) Metode penguapan pelarut, dimana polimer dilarutkan dalam pelarut organik seperti *dichloromethane*, *chloroform* atau *ethyl acetate* yang juga digunakan sebagai pelarut hidrofobik. Campuran polimer dan larutan hormon kemudian diemulsi dalam larutan yang mengandung surfaktan untuk membentuk emulsi minyak dalam air. Setelah pembentukan emulsi stabil, pelarut organik diuapkan dengan mengurangi tekanan atau pengadukan secara terus menerus; dan (2) Metode emulsifikasi spontan, merupakan modifikasi dari metode sebelumnya, dimana pelarut bersama sejumlah kecil pelarut organik yang larut dalam air digunakan sebagai fase minyak. Difusi spontan dari pelarut menyebabkan turbulensi yang mengarah pada pembentukan partikel nano. Peningkatan konsentrasi air yang bercampur dengan pelarut akan menurunkan ukuran partikel (Mohanraj & Chen 2006).

Metode polimerisasi monomer

Metode ini menggunakan senyawa polialkilsianoakrilat (PACA). Metil atau etil sianoakrilat dimasukkan dalam media asam dengan penambahan surfaktan. Monomer sianoakrilat ditambahkan dalam campuran yang sedang diaduk dengan stirer magnetik. Hormon ditambahkan baik sebelum penambahan monomer maupun setelah reaksi polimerisasi. Suspensi partikel nano yang terbentuk dimurnikan dengan ultrasentrifugasi (Soppimath et al. 2001). Dalam metode ini, monomer yang dipolimerisasi untuk membentuk partikel nano berada dalam larutan berair. Hormon dilarutkan pada tahap pertengahan dari polimerisasi atau dengan absorpsi (penempelan) ke dalam partikel nano setelah polimerisasi selesai. Suspensi partikel nano kemudian dimurnikan untuk menghilangkan berbagai stabilisator dan surfaktan yang digunakan pada saat polimerisasi dengan cara ultra sentrifugasi dan menyimpannya dalam media surfaktan yang isotonik (Zhang et al. 2001). Pembentukan nanokapsul dan ukuran partikelnya tergantung dari konsentrasi surfaktan dan stabilisator yang digunakan (Puglisi et al. 1995).

Metode gelasi ionik atau koaservasi polimer hidrofilik

Metode ini tidak memerlukan surfaktan seperti metode polimerisasi monomer. Polimer yang digunakan dalam metode ini merupakan polimer larut air seperti chitosan, natrium alginat dan gelatin. Partikel nano umumnya terbentuk secara spontan ataupun dengan penambahan pengemulsi (Soppimath et al. 2001). Telah banyak penelitian yang memfokuskan pada penyusunan partikel nano menggunakan polimer hidrofilik seperti chitosan (Mao et al. 2001; Feng et al. 2009; Mallick et al. 2012), gelatin (Kommareddy & Amiji 2005; Ethirajan et al. 2008; Gaihre et al. 2009) dan alginat (Sarmento et al. 2006; Finotelli et al. 2010; Martínez et al. 2011). Calvo et al. (1997) telah mengembangkan metode untuk mempersiapkan hidrofilik partikel nano chitosan dengan metode gelasi ionik. Metode ini melibatkan campuran dari dua fase yaitu chitosan polimer dan polianion natrium tripolifosfat. Dalam metode ini, gugus amino bermuatan positif dari chitosan berinteraksi dengan tripolifosfat bermuatan negatif untuk membentuk koaservasi berukuran nano (Gambar 1). Koaservasi terbentuk sebagai hasil interaksi elektrostatik antara dua fase, sedangkan gelasi ionik melibatkan bahan yang mengalami perubahan dari cair menjadi gel karena kondisi interaksi ionik pada suhu kamar.



A B C

A: Sodium tripolifosfat; B: Chitosan; C: Partikel nano chitosan

Gambar 1. Skema diagram struktur fungsional partikel nano chitosan yang terbentuk selama proses gelasi ionik

Sumber: Rather et al. (2013)

Metode fluida

Metode ini menggunakan senyawa yang memiliki suhu dan tekanan di atas titik kritis. Senyawa yang termasuk dalam golongan ini antara lain karbon dioksida, air dan gas metana. Senyawa ini digunakan sebagai pengganti pelarut organik yang berbahaya bagi lingkungan (Soppimath et al. 2001). Metode konvensional seperti ekstraksi-evaporasi pelarut, difusi pelarut dan metode pemisahan fase organik membutuhkan pelarut organik yang berbahaya bagi lingkungan tak terkecuali sistem fisiologis. Metode ini merupakan teknologi alternatif untuk membuat mikro dan partikel nano yang *biodegradable* dan ramah lingkungan. Metode ini secara umum dapat didefinisikan sebagai pelarut yang berada di atas suhu kritis, dimana cairan tetap pada satu fase dan terhindar dari tekanan. *Supercritical* CO_2 (SC CO_2) merupakan fluida yang paling banyak digunakan karena tetap ringan dalam kondisi kritis (T_c = 31,1°C, P_c = 73,8 bar), tidak beracun, tidak mudah terbakar dan harganya murah (Jung & Perrut 2001).

KARAKTERISTIK PARTIKEL NANO

Ukuran partikel

Ukuran partikel dan distribusi ukuran merupakan karakteristik yang paling penting pada sistem partikel nano. Ukuran partikel dipengaruhi oleh jenis dan konsentrasi penstabil, kecepatan homogenisasi dan konsentrasi polimer. Sedangkan untuk menghasilkan ukuran partikel yang kecil dapat menggunakan homogenisasi ultrasonik (Kwon et al. 2001). Ukuran partikel menentukan distribusi secara *in vivo*, toksisitas dan kemampuan pencapaian target secara tepat waktu dan tepat sasaran. Selain itu, ukuran partikel dapat mempengaruhi kapasitas dan pelepasan hormon serta stabilitas partikel nano. Panyam & Labhasetwar (2003) menunjukkan bahwa partikel nano ukuran submikron memiliki keunggulan dibandingkan dengan mikropartikel dalam pemberian hormon. Partikel nano memiliki serapan intraselular yang lebih tinggi dibandingkan dengan mikropartikel dan mencapai target biologis yang lebih luas, dikarenakan ukuran yang kecil dan mobilitas yang stabil. Desai et al. (1997) melaporkan bahwa 100 nm partikel nano memiliki daya serap 2,5 kali lebih besar dari 1 µm mikropartikel dan enam kali lebih besar dari 10 µm mikropartikel dalam sel kanker kolon (*Caco*-2).

Pelepasan hormon dipengaruhi oleh ukuran partikel, dimana partikel yang lebih kecil memiliki luas permukaan yang lebih besar, sedangkan, partikel yang lebih besar memiliki inti yang besar sehingga lebih banyak hormon yang akan dikemas dan pelepasan keluar secara perlahan. Partikel yang lebih kecil memiliki resiko agregasi partikel yang lebih besar selama penyimpanan dan transportasi partikel nano (Redhead et al. 2001).

Sifat permukaan partikel nano hormon

Pemberian partikel nano secara intravena, biasanya mudah dikenali oleh sistem kekebalan tubuh dan kemudian dibersihkan oleh fagosit melalui sirkulasi darah. Selain ukuran partikel nano, permukaan partikel nano yang hidrofobik menentukan jumlah partikel yang diserap oleh komponen darah, terutama protein yang disebut *opsonins* (Brigger et al. 2002). Pengikatan *opsonins* ke permukaan partikel nano disebut opsonisasi, bertindak sebagai jembatan antara partikel nano dan fagosit. Salah satu cara untuk meningkatkan keberhasilan partikel nano mencapai target utama yaitu dengan meminimalkan opsonisasi

dan memperpanjang sirkulasi partikel nano secara *in vivo*. Hal ini dapat dicapai dengan membuat lapisan permukaan partikel nano dengan surfaktan yang hidrofilik dan formulasi partikel nano dengan *copolymers biodegradable* yang hidrofilik seperti *polyethylene glycol*, *polyethylene oxide*, *polyoxamer*, *polyoxamine* dan *polysorbate* 80 (Olivier 2005).

Muatan ion (listrik) permukaan merupakan parameter penting berkaitan dengan stabilitas suspensi dan adhesi partikel dengan permukaan biologis. Dalam mekanisme pembentukan partikel nano chitosan, dimana chitosan sebagai kelompok amina yang bermuatan positif dinetralkan oleh muatan negatif dari molekul tripolifosfat (Urrusuno et al. 1999). Gugus amina residu akan bertanggung jawab atas potensi positif, dimana semakin tinggi potensi zeta dalam rentang waktu tertentu menunjukkan partikel nano chitosan yang lebih stabil. Kelompok rantai amina yang panjang menghambat adsorpsi anion dan menunjukkan pencegahan agregasi (Wang et al. 2008). Potensi zeta dari partikel nano digunakan untuk mengkarakterisasi muatan ion permukaan dari partikel nano. Partikel nano dengan potensi zeta di atas (+/-) 30 mV telah terbukti stabil dalam suspensi dan mampu mencegah agregasi partikel.

Kapasitas muatan ion (listrik)

Keberhasilan sistem partikel nano idealnya harus memiliki kapasitas muatan ion yang tinggi sehingga mengurangi jumlah bahan yang dibutuhkan. Kapasitas muatan ion dapat dilakukan dengan dua metode, yaitu memasukkan hormon bersamaan dengan pembentukan partikel nano (metode penggabungan) dan memasukkan hormon setelah pembentukan partikel nano dengan menginkubasi bahan pembawa di dalam larutan terkonsentrasi (adsorpsi/teknik penyerapan). Kapasitas muatan ion sangat tergantung pada kelarutan hormon di dalam polimer, komposisi polimer, berat molekul, interaksi polimer-hormon dan gugus fungsional (ester atau karboksil) (Panyam et al. 2004). Makromolekul atau protein menunjukkan kapasitas muatan ion yang efisien ketika berada pada titik isoelektrik dengan kelarutan minimal dan adsorpsi maksimum, sedangkan untuk mikromolekul, penggunaan interaksi ionik antara hormon dengan bahan partikel nano menjadi cara yang sangat efektif dalam meningkatkan kapasitas muatan ion (Calvo et al. 1997; Chen et al. 2003).

Pelepasan hormon

Untuk mengembangkan sistem partikel nano yang baik, pelepasan hormon dan polimer yang dapat mengalami biodegradasi merupakan faktor utama yang perlu dipertimbangkan. Secara umum, tingkat pelepasan hormon yang diberikan tergantung pada: (1) Kelarutan hormon; (2) Daya inplasi hormon; (3) Difusi hormon melalui matriks partikel nano; (4) Proses erosi/ degradasi matriks partikel nano; dan (5) Kombinasi proses erosi dan difusi. Jika proses difusi hormon lebih cepat dari erosi, mekanisme pelepasan sebagian besar dikontrol oleh proses difusi. Kejadian pelepasan hormon yang cepat terutama disebabkan oleh kurangnya daya inplasi hormon pada permukaan partikel nano (Magenheim et al. 1993). Hormon yang dimuat melalui metode penggabungan, memiliki efek pelepasan sedikit-sedikit secara berkelanjutan. Jika partikel nano dilapisi oleh polimer, pelepasan hormon secara difusi dari inti membran polimer. Lapisan membran bertindak sebagai penghalang, sehingga kelarutan dan difusi dalam membran menjadi faktor penentu dalam pelepasan hormon (Fresta et al. 1995).

Berbagai metode yang dapat digunakan untuk mempelajari pelepasan hormon secara *in vitro* adalah: (1) Difusi sel dengan membran buatan atau biologis; (2) Dialisis kantong difusi; (3) Agitasi diikuti oleh ultrasentrifugasi; dan (4) Ultrafiltrasi atau sentrifugal ultrafiltrasi. Metode yang umum dilakukan adalah kontrol agitasi yang diikuti dengan sentrifugasi, namun karena prosesnya membutuhkan waktu yang relatif lama dan adanya faktor kesulitan dalam pemisahan partikel nano dari media pelepasan, maka teknik dialisis lebih disukai (Mohanraj & Chen 2006).

Beberapa hasil penelitian aplikasi penggunaan partikel nano dalam pemanfaatan berbagai hormon berkaitan dengan metode dan komponen utama yang digunakan serta karakteristik partikel nanonya dapat dilihat pada Tabel 1.

PERANAN CHITOSAN SEBAGAI KOMPONEN UTAMA PEMBUATAN PARTIKEL NANO

Chitosan merupakan polimer alami dari sumber daya terbarukan, yang diperoleh dari cangkang kerang, kulit udang dan limbah industri makanan laut dengan struktur molekul menyerupai serat pada sayuran dan buah-buahan (Kim et al. 2008). Chitosan merupakan polisakarida kationik yang diperoleh dari deasetilasi chitin yang memiliki sifat unik seperti biokompatibilitas, biodegradabilitas, imunogenisitas yang rendah dan nontoksis serta melibatkan hidrolisis basa (Thanou et al. 2001). Chitosan dapat digunakan dalam persiapan partikel nano untuk implan hormon melalui parenteral, hidung, mata dan transdermal (Wang et al. 2007) dengan keuntungan harga murah, umur simpan panjang dan kapasitas tampung hormon yang besar (Janes et al. 2001).

Chitosan telah digunakan sebagai eksipien farmasi dalam formulasi hormon yang sukar larut dan untuk mendapatkan pelepasan hormon yang terkontrol

(Krauland et al. 2006). Chitosan juga memiliki potensi besar untuk sistem pengiriman hormon melalui hidung, memfasilitasi lewatnya molekul hidrofilik yang besar (seperti salmon kalsitonin dan insulin) melalui mukosa hidung dengan sirkulasi yang sistemik (Ilium et al. 1994). Beberapa keuntungan chitosan dalam pengembangan partikel nano diantaranya: (1) Kemampuan untuk mengontrol pelepasan zat aktif; (2) Menghindari penggunaan pelarut organik berbahaya ketika partikel terlarut dalam larutan asam; (3) Poliamina linear berisi sejumlah kelompok amina bebas; (4) Sifat kationik yang memungkinkan untuk silang ionik dengan anion *multivalent*; (5) Memiliki karakter mukoadhesif yang meningkatkan waktu penyerapan; dan (6) Stabilitas yang baik (Wang et al. 2008; Lee et al. 2011).

PEMBERIAN PARTIKEL NANO HORMON MELALUI PENCIUMAN

Dalam beberapa tahun terakhir, aplikasi pemberian material hormon melalui hidung telah menjadi perhatian dikarenakan metodenya yang mudah dan dapat diandalkan. Pemberian hormon melalui hidung merupakan cara alternatif untuk menargetkan hormon langsung ke otak melalui syaraf penciuman (Westin et al. 2005). Faktor pembatas pemberian material melalui hidung adalah cairan mukosa hidung dan permeabilitasnya yang rendah sehingga membatasi penyerapan material. Namun, polimer bioadhesif dapat digunakan untuk meningkatkan waktu keberadaan material di hidung yang memungkinkan penyerapan lebih lama (Critchley et al. 1994). Chitosan partikel nano dapat mengikat dengan kuat bahan yang bermuatan negatif seperti permukaan sel dan cairan mukosa. Cairan mukosa mengandung mucin yang memiliki konstitusi kimia yang berbeda, namun beberapa mengandung proporsi yang signifikan dari asam *sialic*. Pada kondisi pH fisiologis, asam *sialic* membawa muatan negatif yang mengakibatkan interaksi elektrostatik yang kuat antara mucin dan chitosan dalam larutan (Soane et al. 1999).

Penggunaan chitosan tidak hanya untuk meningkatkan adhesi antara formulasi dan jaringan hidung, tetapi juga pada proses transportasi *paracellular* (Dodane et al. 1999). Studi immuno histologis menunjukkan bahwa chitosan dapat membuka hubungan antara sel-sel yang terlalu ketat melalui efek filamen factin. Chitosan tidak mengandung racun, lebih toleran, serta efek kombinasi bioadhesi dan transportasi *paracellular* menjadi bahan pertimbangan pemberian estradiol melalui rongga hidung (Wang et al. 2008).

APLIKASI PARTIKEL NANO DALAM PEMANFAATAN HORMON

Aplikasi nanoteknologi sudah berkembang sangat pesat dan digunakan dalam berbagai keperluan, namun aplikasinya dalam pemanfaatan hormon masih sangat terbatas, apalagi kaitannya dengan hormon reproduksi. Rather et al. (2013) menggunakan ukuran partikel chitosan dan chitosan *gold* partikel nano hormon LHRH sebesar 192,5±19,1 dan 114±10,3 nm. Kedua partikel memiliki struktur yang kompak berbentuk bola, tersebar merata dan stabil dengan *polydispersity index* 0,33-0,47 dan potensi zeta -33,14 hingga -34,95 mV (Tabel 1). Efisensi penjebakan hormon LHRH dari chitosan partikel nano sebesar 69%, sedangkan chitosan *gold* partikel nano sebesar 60%. Selanjutnya mereka membandingkan antara ikan salmon betina yang di suntik secara intramuskular dengan chitosan hormon LHRH dosis 0,2 ml/kg bobot badan dan chitosan *gold* partikel nano hormon LHRH dosis 0,1 ml/kg bobot badan. Hasil penelitian menunjukkan bahwa chitosan dan chitosan *gold* partikel nano hormon LHRH yang disuntikkan efektif melepaskan hormon secara berkelanjutan selama 24 jam. Lonjakan hormon LHRH mencapai puncaknya tujuh jam setelah penyuntikan dan menurun tajam setelahnya. Total telur yang dihasilkan 130 dan 67% lebih tinggi, telur yang dibuahi 17 dan 88% lebih tinggi dan tingkat fertilitasnya 13 dan 9% dibandingkan dengan kontrol pada masing-masing chitosan dan chitosan *gold* partikel nano hormon LHRH.

Wang et al. (2008) melakukan penelitian pada partikel nano chitosan hormon estradiol (E_2) yang bertujuan untuk meningkatkan bioavalabilitas E_2 dengan target sasaran pada otak dengan pemberian melalui mukosa hidung. Mereka membuat chitosan partikel nano E_2 dengan metode gelasi ionik chitosan menggunakan anion tripolifosfat (TPP) diperoleh ukuran partikel rata-rata 269,3±31,6 nm, potensi zeta +25,4 mV, kapasitas muatan 1,9 mg/ml, rata-rata efisiensi pengikatan sebesar 64,7%. Selanjutnya, chitosan partikel nano E_2 diberikan kepada tikus jantan melalui intranasal dan intravena dengan dosis 0,48 mg/kg. Kadar E_2 plasma darah dicapai setelah pemberian intranasal (32,7±10,1 ng/ml) secara signifikan lebih rendah dibandingkan dengan pemberian intravena (151,4±28,2 ng/ml), sedangkan konsentrasi E_2 cairan pada *cerebrospinal* (*cerebrospinal fluid*/CSF) dicapai setelah pemberian intranasal (76,4±14 ng/ml) secara signifikan lebih tinggi dibandingkan dengan pemberian intravena (29,5±7,4 ng/ml). *Drug target index* (DTI) ketika

Tabel 1. Karakteristik partikel nano dan aplikasinya dalam pemanfaatan hormon

Hormon	Metode	Komponen utama	Ukuran partikel (nm)	Potensi zeta (mV)	PDI	Kapasitas muatan (mg/ml)	Efisiensi penjebakan (%)	Referensi
LHRH	Gelasi ionik	Chitosan	114,00±10,30	-33,14±6,67	0,335	-	69,00	Rather et al. (2013)
		Chitosan *gold*	192,50±19,10	-34,95±7,50	0,470	-	60,00	
Estradiol	Gelasi ionik	Chitosan	269,30±31,60	+25,40	-	1,90	64,70	Wang et al. (2008)
Estradiol	Penguapan pelarut	PLGA	186,00±10,00	-	0,127	-	55,20±1,00	Esmaeili et al. (2008)
Estradiol	Penguapan pelarut	EA/DMAB	116,00±2,60	-	-	-	-	Sahana et al. (2008)
		DCM: EA 70:30/DMAB	253,00±5,50	-	-	-	-	
		EA/PVA	279,30±2,50	-	-	-	-	
Insulin	Gelasi ionik	Chitosan	243,00±15,00		0,500		62,99±1,67	Ma et al. (2002)
Insulin	Penguapan pelarut	PNP	150,00±17,00	-	-	-	50,30±3,10	Cui et al. (2007)
		PHNP	169,00±16,00	-	-	-	65,41±2,30	

LHRH: *Luteinizing hormone releasing hormone*; PDI: *Polydispersity index*; DMAB: *Didodecyldimethyl amonium bromide*; DCM: diklorometana; PVA: *Polivinil alcohol*; EA: Etil asetat; PNP: *Poly lactide-co-glycolide*; PHNP: *Poly lactide-co-glycolide-Hp55*; PLGA: *Poly lactide-co-glycolide*

berada di hidung sebesar 3,2 dengan persentase E_2 mencapai target sebesar 68,4%. Hasil ini menunjukkan bahwa E_2 dapat langsung diangkut dari rongga hidung ke dalam cairan CSF pada tikus. Partikel nano chitosan E_2 telah terbukti efektif dalam pengobatan berbagai penyakit seperti Hiperlipidemia (Mittal et al. 2007) dan Alzheimer (Wang et al. 2008).

Mittal et al. (2011) mengembangkan Tween 80 (T-80) yang dilapisi *polylactide-co-glycolide* (PLGA) dalam membentuk partikel nano E_2. Estradiol yang mengandung partikel nano dibuat dengan metode emulsifikasi spontan dan lapisan T-80 dicapai dengan menginkubasi ulang partikel nano pada konsentrasi T-80 yang berbeda. Partikel nano E_2 kemudian diuji coba pada tikus melalui pemberian secara oral. Hasil penelitian menunjukkan bahwa konsentrasi partikel nano E_2 yang dilapisi T-80 mengalami peningkatan dari 9,72±1,07 mg/ml menjadi 63,84±3,59 mg/ml pada peningkatan konsentrasi awal T-80 dari 1 menjadi 5% dengan kondisi yang stabil dalam cairan lambung dan usus. Pemberian partikel nano E_2 yang dilapisi T-80 secara oral mengakibatkan konsentrasi E_2 pada otak secara signifikan lebih tinggi setelah 24 jam dibandingkan dengan kontrol dan mencegah ekspresi amiloid beta-42 (Aβ42) pada otak.

Sahana et al. (2008) melakukan penelitian yang bertujuan untuk mengoptimalkan ukuran partikel dan efisiensi pengikatan dari partikel nano E_2 dengan pelarut yang berbeda. Pembentukan partikel nano dengan metode penguapan pelarut menggunakan *didodecyldimethylammonium bromide* (DMAB) atau polivinil alkohol (PVA) sebagai stabilisator. Etil asetat (EA), aseton (ACE), kloroform (CHL), dan diklorometana (DCM) digunakan sebagai pelarut organik baik secara individu atau dalam kombinasi. DMAB bila digunakan sebagai surfaktan menghasilkan ukuran partikel yang lebih kecil dibandingkan dengan PVA terlepas dari pelarut organik yang digunakan. Di sisi lain, PVA menghasilkan partikel dengan efisiensi penjebakan yang lebih tinggi ketika menggunakan kombinasi pelarut organik. Kombinasi DCM dengan EA menghasilkan efisiensi penjebakan tertinggi pada kedua stabilisator. bioavalabilitas dari partikel nano E_2 yang diberikan pada tikus dengan dosis 1 mg/ekor diperoleh bahwa EA/DMAB (ukuran partikel 116,0±2,6 nm) dan DCM:EA = 70:30/DMAB (ukuran partikel 253,0±5,5 nm) menunjukkan rilis E_2 selama sembilan dan lima hari, sedangkan EA/PVA (ukuran partikel 279,3±2,5 nm) dengan rilis E_2 selama tiga hari.

Cui et al. (2007) melakukan penelitian penggunaan *poly lactide-co-glycolide* partikel nano (PNP) dan *poly lactide-co-glycolide*-Hp55 partikel nano (PHNP) sebagai pembawa hormon insulin dalam rangka mengurangi kadar glukosa pada serum. Pembentukan partikel nano dilakukan menggunakan metode penguapan pelarut. Ukuran partikel dari PNP dan PHNP berturut-turut sebesar 150±17 dan 169±16 nm dengan *drug recovery* masing-masing sebesar 50,30±3,1 dan 65,41±2,3%. Pelepasan hormon insulin dari partikel nano dalam cairan lambung setelah satu jam berturut-turut sebesar 50,46±6,31 dan 19,77±3,15%. Bioavailabilitas dari PNP dan PHNP insulin dengan pemberian secara subkutan (dosis 1 IU/kg) pada tikus sebesar 3,68±0,29 dan 6,27±0,42%. Hasil penelitian menunjukkan bahwa penggunaan PHNP insulin merupakan metode yang efektif untuk mengurangi kadar glukosa pada serum.

KEKURANGAN DAN EFEK NEGATIF PARTIKEL NANO HORMON

Aplikasi nanoteknologi pada beberapa dekade ini berkembang sangat pesat dan digunakan dalam berbagai keperluan. Ukuran partikel yang kecil dengan efisiensi yang lebih tinggi merupakan alasan utama teknologi ini dikembangkan. Namun, ternyata tidak hanya efek positif yang dihasilkan tetapi juga kekurangan dan efek negatifnya. Partikel nano memiliki kekurangan, diantaranya sulit dalam penanganan dan penyimpanan karena mudah teragregasi, tidak cocok digunakan untuk hormon dosis tinggi, ukurannya yang kecil menyebabkan partikel nano mudah menembus membran inti sel sehingga menyebabkan mutasi genetik yang tidak diinginkan (Rawat et al. 2006).

Partikel nano dapat memasuki tubuh manusia melalui berbagai macam mekanisme. Kontak langsung partikel nano dapat membahayakan kesehatan manusia, antara lain dapat mengganggu jalannya transportasi substansi vital masuk dan keluar sel, sehingga dikhawatirkan efek jangka panjangnya dapat berakibat pada gangguan fisiologis dan fungsi sel secara normal. Partikel nano terlebih dahulu disimpan di dalam vesikel yang berada pada permukaan sel. Vesikel-vesikel kecil kemudian bergabung membentuk vesikel besar seperti badan multivesikular. Badan multivesikular ini kemudian bergabung dengan lisosom, dimana protein dan makromolekul lainnya dipecah oleh protease dan enzim lainnya. Partikel nano hormon yang terkandung di dalamnya dapat menyebar di dalam sel dan dapat keluar melalui jalur endosom (Iversen et al. 2011).

Efek samping dari paparan partikel nano tidak dapat dihindari. Paparan partikel nano dapat mempengaruhi saluran pencernaan bagian atas sebelum partikel mencapai paru-paru, lambung dan usus, tergantung dari ukuran, konsentrasi dan jenis partikel, serta lamanya kontak. Contohnya pada introduksi partikel nano secara oral atau penciuman, efek dari eksposisi partikel nano pada sel-sel mukosa rongga mulut dan hidung, ditandai dengan adanya peradangan dan alergi pada sel selaput lendir hidung (Aust et al. 2009). Walaupun sudah diuraikan kekurangan dan efek

negatif dari partikel nano, studi-studi yang lebih mendalam diperlukan untuk membuktikan dan menghambat efek-efek negatif yang mungkin terjadi.

KESIMPULAN

Secara umum pembentukan partikel nano hormon menggunakan polimer berhasil baik dengan distribusi massa molekul yang baik dan stabil, sehingga dapat digunakan sebagai komponen pembawa hormon dengan tetap mempertimbangkan adanya efek negatif. Partikel nano memiliki potensi yang besar sebagai komponen pembawa hormon karena kemampuannya dalam mempertahankan kinerja hormon melalui perlindungan stabilitas hormon dalam perjalanan dari fagosit dan berguna dalam pelepasan secara terkontrol dalam waktu lama di dalam tubuh, sehingga dapat mencapai organ target dengan baik. Walaupun demikian, perlu diingat bahwa partikel nano dapat masuk ke dalam tubuh organisme hidup dengan berbagai jalan. Di dalam tubuh, partikel nano dapat menyebar melalui ruang dalam sel dan masuk ke dalam inti sel. Oleh karena itu, gunakanlah bahan partikel nano dengan efek minimal pada kesehatan manusia.

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WARTAZOA Vol. 25 No. 4 Th. 2015 Hlm. 181-188 DOI: http://dx.doi.org/10.14334/wartazoa.v25i4.1228

Upaya Pengembangan Teknologi Cepat Transkripsi dan Translasi *In Vitro* dalam Sintesis Vaksin di Indonesia

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(Diterima 6 April 2015 – Direvisi 19 Oktober 2015 – Disetujui 23 Oktober 2015)

ABSTRAK

Produksi protein fungsional (termasuk vaksin) menggunakan teknik konvensional dengan telur ayam berembrio memerlukan tenaga dan waktu lebih lama. Penggunaan teknik sintetik secara kimiawi dinilai tidak praktis untuk menghasilkan peptida yang berukuran lebih panjang dari 20 residu. Sebaliknya, teknologi transkripsi dan translasi *in vitro* dapat menggunakan produk *polymerase chain reaction* (PCR) secara langsung sebagai *template* untuk menghasilkan vaksin hanya dalam waktu dua jam dengan ketepatan tinggi. Teknik ini bahkan mampu menghasilkan protein 1-10 mg/ml lebih tinggi dibandingkan dengan teknik konvensional yang hanya menghasilkan satu dosis per butir telur. Pada tinjauan ini dibahas keuntungan dan kerugian produksi vaksin hewan dengan teknologi konvensional, serta transkripsi dan translasi *in vitro*. Beberapa kelebihan teknologi transkripsi dan translasi *in vitro* menyebabkan teknologi tersebut dapat dipertimbangkan sebagai metode cepat untuk skrining vaksin maupun untuk menghasilkan vaksin baru bagi hewan.

Kata kunci: Vaksin, transkripsi, translasi, *in vitro*

ABSTRACT

Efforts to Develop Rapid Technology of *In Vitro* Transcription and Translation in Vaccine Synthesis in Indonesia

Production of functional protein (including vaccine) using conventional technology in embryonated chicken eggs is laborious and lengthy. The use of chemical synthesis is not practical for peptides longer than 20 residues. In contrast, *in vitro* transcription and translation technology can directly utilize polymerase chain reaction (PCR) product as template for vaccine synthesis within two hours accurately. Moreover, up to 1-10 mg/ml protein can be produced using the technology compared to conventional method that only gives approximately one dose per egg. In this review, advantages and disadvantages of animal vaccine generation using conventional and *in vitro* methods would be described. *In vitro* transcription and translation technology can be considered as the most practical and efficient technique for rapid screening and generating new animal vaccines.

Key words: Vaccine, transcription, translation, *in vitro*

PENDAHULUAN

Sampai saat ini, vaksinasi masih diyakini sebagai cara yang paling murah dan efektif untuk mencegah serangan penyakit tertentu baik pada hewan maupun manusia. Hal ini disebabkan karena penggunaan vaksin dapat menimbulkan kekebalan aktif yang spesifik di dalam tubuh sehingga terjadinya serangan penyakit tertentu dapat ditangkal secara internal. Tindakan ini menjadi pilihan utama untuk pengobatan yang tidak hanya mahal tapi juga sering tidak efektif karena agen penyakit sudah terlanjur menginfeksi.

Lu et al. (2014) melaporkan bahwa vaksinasi merupakan cara yang paling efektif untuk menaggulangi penyakit flu burung yang sering sekali menjadi ancaman pada ternak unggas maupun manusia. Tingkat mortalitas virus H5N1 secara global pada manusia yang mencapai 75% pada tahun 2008 perlu diwaspadai. Penyakit yang dikhawatirkan merupakan ulangan dari pandemik influenza yang terjadi pada tahun 1918 tersebut telah menelan korban hingga 50 juta orang di seluruh dunia.

Sejak Edward Jenner menemukan vaksin cacar pada tahun 1796 yang diujicobakan pada seorang pemuda pemberani bernama James Phipps, penggunaan sumber patogen yang telah dilemahkan (atenuasi) menjadi cara paling mudah untuk menghasilkan bahan yang dipergunakan sebagai vaksin. Penemuan vaksin tersebut kemudian dilanjutkan oleh Louis Pasteur yang berhasil menemukan vaksin kolera dan rabies. Zichel et al. (2010) menyatakan bahwa vaksin tradisional generasi pertama dihasilkan melalui beberapa tahapan perlakuan patogen dengan formaldehida. Penggunaan beberapa bahan kimia, seperti β-*propriolactine* maupun kloroform, juga dapat menghilangkan patogenitas tanpa menghilangkan sifat antigenisitasnya (kemampuan untuk memicu respon kekebalan yang diinginkan). Namun, penggunaan vaksin yang diperoleh dengan

tehnik tersebut mulai ditinggalkan karena kekhawatiran terhadap kemampuan patogen untuk melakukan infeksi.

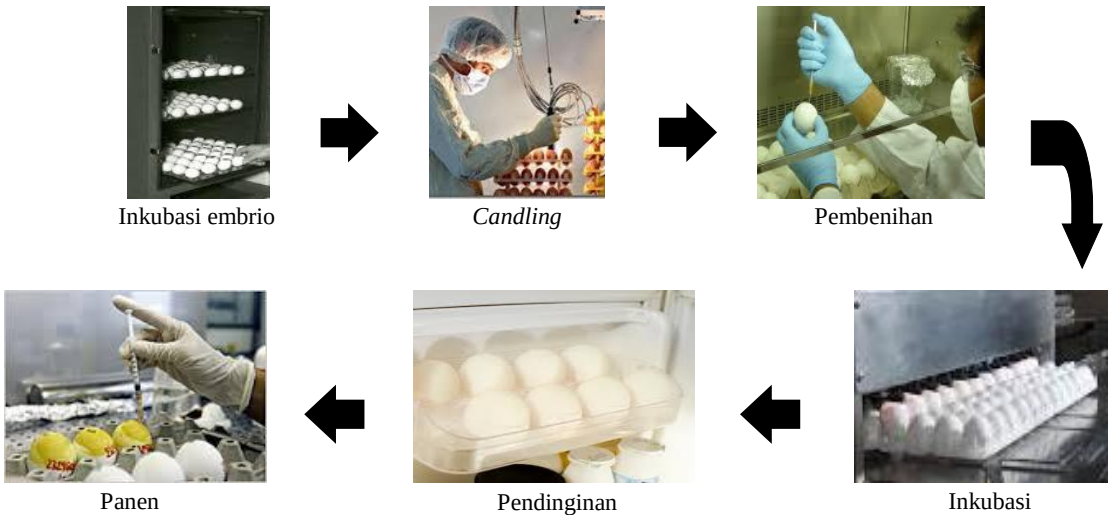
Sampai tahun 2007, semua vaksin influenza komersial dihasilkan dari telur ayam berembrio. Namun, Kasson & Pande (2008) melaporkan bahwa H1N1 yang dihasilkan pada telur ayam berembrio belum mampu merangsang kekebalan tubuh yang cukup sehingga diperlukan dosis yang lebih banyak. Selain itu, untuk menghasilkan vaksin dengan metode tersebut dibutuhkan waktu yang cukup lama, yaitu sekitar 6-8 bulan. Kondisi tersebut tentu akan menjadi hambatan terutama ketika terjadi wabah yang meluas (pandemik) (Bommakanti et al. 2010). Kasson & Pande (2008) menambahkan bahwa vaksin yang dihasilkan dari telur ayam berembrio tersebut tidak dapat diandalkan untuk mengatasi pandemik influenza yang lebih ganas seperti H5N1.

Seiring dengan kemajuan biologi molekuler, serta telah ditemukannya urutan basa (sekuen) genom dari aneka jenis patogen maka penggunaan teknologi DNA rekombinan dapat dipakai untuk menghasilkan protein fungsional yang dapat berfungsi sebagai bahan vaksin. Teknologi tersebut telah memungkinkan dihasilkannya protein tertentu dengan menggunakan gen pengkode dari patogen. Melalui kloning gen pengkode protein tertentu dari patogen yang kemudian dikombinasi dengan DNA pembawa (plasmid), maka DNA rekombinan tersebut dapat dimasukkan ke sel inang untuk menghasilkan vaksin yang diinginkan. Produk protein tertentu yang berasal dari patogen (tanpa unsur genom patogen tersebut di dalamnya) akan menghasilkan vaksin yang lebih aman daripada jenis vaksin yang dihasilkan dari patogen yang dilemahkan (Wong & Webby 2013).

PRODUKSI VAKSIN MENGGUNAKAN TEKNOLOGI KONVENSIONAL

Produksi vaksin dengan teknologi konvensional dilakukan pada telur ayam yang memiliki embrio (*embryonated chicken egg*). Produksi vaksin dengan teknik ini terdiri dari beberapa tahap, dimulai dengan proses pembentukan embrio pada telur melalui inkubasi sampai embrio berumur 9-11 hari. Proses *culling* dilakukan terhadap telur-telur infertil melalui proses peneropongan dengan lampu (*candling*). Setelah itu, virus penyebab penyakit tertentu disuntikkan ke dalam cairan allantoik telur setelah terlebih dahulu dilakukan sterilisasi cangkang telur menggunakan 75% etanol. Proses inkubasi dilakukan selama 2-4 hari pada suhu 33-36°C kemudian di-*candling* untuk memisahkan telur yang embrionya mati. Setelah itu, telur didinginkan di kulkas untuk kemudian dilakukan panen cairan allantoik, penonaktifan secara kimiawi, serta pemisahan cairan tersebut dari protein-protein telur dengan filterisasi ataupun sentrifugasi. Proses ini diakhiri dengan uji antigenisitas vaksin dengan vaksin standar atau virus asli sebagai kontrol positif (Wong & Webby 2013). Tahapan produksi vaksin menggunakan telur ditampilkan pada Gambar 1.

Produksi vaksin dengan teknik konvensional di atas memiliki banyak kelemahan, diantaranya rendahnya kuantitas vaksin yang dihasilkan, lama waktu yang dibutuhkan, memerlukan banyak tahapan yang memerlukan tenaga dan keahlian khusus, serta peluang terjadinya kontaminasi dengan mikroorganisme lain (Wong & Webby 2013; Lu et al. 2014). Untuk menghasilkan satu dosis vaksin diperlukan 1-2 ekor butir telur ayam. Pada tahun 2002, kebutuhan dunia terhadap vaksin influenza diperkirakan mencapai 1.239



Gambar 1. Proses produksi vaksin menggunakan telur

juta dosis per tahun, sehingga diperlukan jumlah telur ayam yang sangat besar dengan pekerjaan yang cukup berat (Adu-Bobie et al. 2002). Lebih lanjut dijelaskan bahwa untuk mengatasi terjadinya pandemik, diperlukan sekitar 5-10 kali lipat dari jumlah tersebut dalam setiap tahun.

Untuk mengatasi kelemahan produksi vaksin dengan teknik di atas, maka telah dihasilkan vaksin subunit sebagai vaksin generasi kedua dengan menggunakan inang. Aneka jenis inang yang digunakan untuk menghasilkan vaksin saat ini diantaranya adalah bakteri, ragi, jamur, alga bersel satu, tanaman, sel serangga maupun sel mamalia (Valdés et al. 2003). Masing-masing inang tersebut memiliki kelebihan dan kelemahan masing-masing tergantung dari jenis dan karakteristik vaksin yang akan dihasilkan. Jika vaksin tersebut membutuhkan proses-proses modifikasi pasca-translasi, maka penggunaan sel prokariotik kurang menguntungkan. Sebaliknya, jika struktur protein vaksin lebih sederhana dan tidak memerlukan modifikasi setelah proses translasi, maka penggunaan prokariotik merupakan pilihan yang paling murah dan praktis (Sahdev et al. 2008).

Diantara inang dari jenis prokariotik, *Escherichia coli* merupakan pilihan yang paling banyak dipakai saat ini. Hal ini disebabkan beberapa alasan, diantaranya karena cepat tumbuh, memerlukan media yang murah, serta genom bakteri tersebut sudah dipelajari secara luas (Sezonov et al. 2007). Metode ini sangat populer akhir-akhir ini karena teknologinya sudah mapan, didukung oleh alat dan bahan yang tersedia secara komersial dan dapat menghasilkan vaksin dalam waktu yang relatif singkat (12 jam) (Ali et al. 2005b). Namun, metode ini juga memiliki kelemahan diantaranya: (1) Memerlukan tahap kloning dan sekuensing; (2) Membutuhkan beberapa enzim untuk pemotongan (restriksi), penyambungan (ligasi), serta untuk amplifikasi berharga mahal; (3) Memerlukan penambahan bahan-bahan induksi untuk ekspresi protein (seperti *isopropyl* β-*D-1-thiogalactopyranoside* /IPTG); (4) Memerlukan peralatan khusus untuk memecah dinding sel bakteri inang (sonikator); (5) Vaksin yang dihasilkan tercampur dengan protein bakteri inang sehingga membutuhkan proses pemurnian; (6) Jumlah vaksin yang dihasilkan sangat tergantung dari kondisi pertumbuhan bakteri yang menjadi inang; (7) Dibutuhkan nutrisi yang lebih banyak karena selain diperlukan untuk membuat vaksin tersebut juga untuk memelihara kelangsungan hidup inang; dan (8) Sulit digunakan untuk menghasilkan vaksin yang beracun, karena dapat membunuh inang penghasil (Ali et al. 2005a; 2005b; 2005c).

TEKNOLOGI TRANSKRIPSI DAN TRANSLASI *IN VITRO*

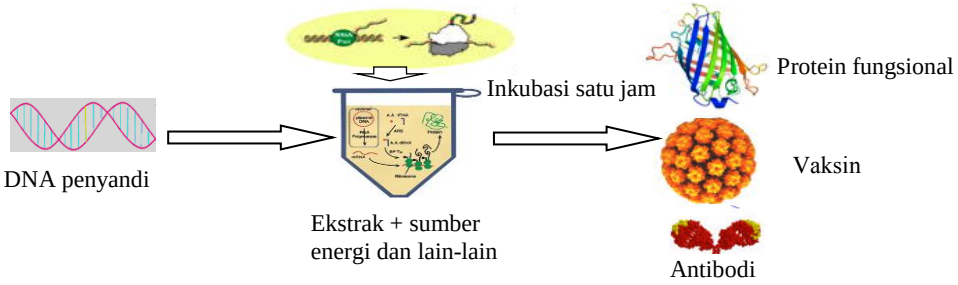
Teknologi transkripsi dan translasi *in vitro* dikenal dengan banyak nama, diantaranya teknik ekspresi protein *in vitro*, sistem ekspresi tanpa sel (*cell-free expression system*), maupun sistem sintesis protein tanpa sel (*cell-free protein synthesis*) (Nakano et al. 2000; Spirin & Swartz 2004; Yang et al. 2004; Ali et al. 2005a).

Teknologi ini menghasilkan protein fungsional dengan menggunakan ekstrak tanaman ataupun bakteri (yang mengandung ribosom dan organel-organel sel lainnya termasuk faktor inisiasi maupun elongasi yang diperlukan untuk sintesis antigen/vaksin), ditambah *transfer ribonucleic acid* (tRNA) yang telah dimurnikan, plasmid (yang telah diperkaya dengan sekuen-sekuen yang berguna untuk mempercepat proses transkripsi translasi serta membawa gen penyandi vaksin yang akan dibuat), enzim RNA polymerase, sumber energi dan enzim pemecah sumber energi (Spirin & Swartz 2004; Yang et al. 2004; Yin & Swartz 2004). Perkembangan terkini dari teknologi tersebut telah memungkinkan penggunaan gen produk *polymerase chain reaction* (PCR) langsung sebagai pengganti plasmid, sehingga proses kloning yang memerlukan waktu dan biaya dapat dihindari (Nakano & Yamane 2004).

Melalui inkubasi bahan-bahan yang diperlukan untuk sintesis protein di atas dan penambahan beberapa *chaperon* (Spirin & Swartz 2004) pada suhu yang sesuai untuk pertumbuhan tanaman ataupun bakteri (25-37°C) selama satu jam, maka vaksin target dapat diperoleh secara langsung (Ali et al. 2005a). Untuk mempermudah pemahaman terhadap sistem teknologi transkripsi dan translasi *in vitro*, diagram sistem tersebut ditampilkan pada Gambar 2.

Berhubung proses tersebut tidak melibatkan makhluk hidup, maka produk tunggal yang dihasilkan tidak tercampur dengan berbagai protein seperti jika menggunakan teknik *in vivo* dengan bakteri, tumbuhan, maupun hewan. Selain itu, karena tidak ada pembatas antara mata ataupun tangan kita dengan reaksi, manipulasi reaksi untuk meningkatkan kuantitas maupun kualitas vaksin dapat dilakukan dengan mudah (Spirin & Swartz 2004; Ali et al. 2005a).

Sebagai sumber ekstrak, tanaman yang sering digunakan adalah biji gandum yang baru tumbuh (Kawasaki & Endo 2004; Tsuboi et al. 2008), *lysat* retikulosit kelinci (Spirin & Swartz 2004), serangga (seperti ulat sutera) dan bakteri *E. coli* (Ali et al.



Gambar 2. Diagram teknologi transkripsi dan translasi *in vitro*

2005a). Penggunaan sumber ekstrak di atas tergantung dari jenis dan struktur protein fungsional yang akan dihasilkan.

Kelebihan produksi protein fungsional dengan teknik transkripsi dan translasi *in vitro* adalah: (1) Waktu singkat (satu jam) (Yin & Swartz 2004); (2) Dapat menggunakan produk PCR sebagai *template* (Nakano et al. 2000); (3) Tidak memerlukan tahapan kloning dan pengecekan hasil cloning; (4) Dapat dilakukan rekayasa untuk mengembangkan fungsi dan meningkatkan aktivitas protein target (Ali et al. 2005a); (5) Menggunakan tanaman/bakteri yang mati sehingga nutrisi hanya dipakai untuk memproduksi vaksin; (6) Dapat dipergunakan untuk produksi vaksin yang beracun bagi inang; (7) Dapat dilakukan optimalisasi terhadap reaksi untuk meningkatkan jumlah vaksin yang diperoleh; dan (8) Berupa *bypass* terhadap hampir semua proses biologis sehingga dapat diaplikasikan dengan teknik robotik otomatis.

PRODUKSI VAKSIN MENGGUNAKAN TEKNOLOGI CEPAT TRANSKRIPSI DAN TRANSLASI *IN VITRO*

Untuk mengatasi kesulitan produksi vaksin dengan menggunakan inang yang hidup, penggunaan sistem transkripsi dan translasi *in vitro* merupakan pilihan yang sangat menarik. Yang et al. (2004) melaporkan telah dihasilkannya protein vaksin untuk limpoma sel B secara cepat melalui penggunaan teknologi transkripsi dan translasi *in vitro*. Penggunaan teknologi tersebut dapat diperbesar skalanya untuk menghasilkan vaksin sesuai kebutuhan.

Tersedianya sekuen genom dari berbagai jenis sumber patogen saat ini memudahkan dilakukannya penelusuran pada berbagai jenis vaksin baru (Adu-Bobie et al. 2002). Eksplorasi vaksin dengan memanfaatkan sekuen genom tersebut disertai oleh studi genomik fungsional memunculkan istilah baru di dunia vaksin, yaitu vaksinologi terbalik (*reverse vaccinology*). Gat et al. (2007) telah memanfaatkan sistem tersebut untuk menghasilkan vaksin dan antibodi terhadap penyakit *anthrax*.

Goerke et al. (2008) melaporkan keberhasilan produksi vaksin dengan menggunakan teknologi *in vitro* dalam skala reaksi 30-40 ml. Lebih lanjut dilaporkan bahwa vaksin yang dihasilkan dengan teknologi tersebut dapat meningkatkan daya tahan (*survival rate*) mencit sampai 20-30% ketika diuji tantang dengan tumor ganas. Berdasarkan hasil penelitian di atas dapat disimpulkan bahwa teknologi tersebut telah berhasil digunakan untuk membuat vaksin aktif yang kuantitas dan kualitasnya tidak berbeda dengan vaksin yang dibuat secara konvensional.

Vaksin rekombinan terhadap botulinum telah dihasilkan dengan menggunakan teknologi transkripsi dan translasi *in vitro* (Zichel et al. 2010). Berkat teknologi tersebut, berbagai macam modifikasi untuk meningkatkan kualitas maupun kuantitas vaksin tersebut dapat dilakukan dengan mudah, diantaranya seperti penambahan enzim disulfide isomerase (PDI) yang ditujukan untuk meningkatkan bentuk alami dari vaksin sehingga jumlah vaksin yang dapat dihasilkan dapat mencapai 1 mg/ml.

Penggunaan teknologi *in vitro* ini masih difokuskan untuk mendapatkan cara menghasilkan vaksin tercepat dengan kemurnian dan kualitas terbaik. Penelitian menyangkut penggunaan vaksin yang dihasilkan langsung kepada manusia belum dilaporkan. Kelemahan vaksin yang dihasilkan dengan teknik ini adalah kuantitas vaksin yang dihasilkan sedikit, karena terjadi degradasi oleh enzim protease dari sisa ekstrak yang digunakan (Ali et al. 2005b). Untuk mengatasi hal ini, beberapa upaya telah dilakukan diantaranya penggunaan ekstrak dari bakteri *E. coli* yang telah dimutasi gen-gen penyandi enzim proteasenya (Ali et al. 2005c).

UPAYA MENINGKATKAN KUALITAS VAKSIN

Keterbatasan penggunaan teknologi transkripsi dan translasi *in vitro* untuk menghasilkan vaksin dan protein fungsional lainnya adalah terjadinya pemecahan (degradasi) vaksin oleh enzim protease yang terdapat pada ekstrak tanaman ataupun ekstrak bakteri yang digunakan (Ali et al. 2005b). Enzim-enzim protease yang masih terdapat pada ekstrak tersebut akan memecah protein-protein rekombinan yang dihasilkan, sehingga jumlah vaksin yang dihasilkan menurun.

Enzim protease yang bersifat degradatif tersebut antara lain enzim protease yang disandi oleh gen-gen Lon, ClpP, DegP dan OmpT (Ali et al. 2005b; 2005c). Gen Lon dan ClpP merupakan gen penyandi enzim protease serin dari *E. coli* yang terdapat pada sitoplasma. Gen DegP dan OmpT masing-masing merupakan gen penyandi enzim protease serin dari *E. coli* yang terdapat pada periplasma dan membran luar (*outer-membrane*). Untuk itu, penggunaan ekstrak bakteri mutan yang tidak memiliki enzim tersebut diduga akan menghasilkan antibodi dalam jumlah yang lebih banyak.

Untuk membuktikan hipotesis di atas, (Ali et al. 2005c) telah memodifikasi gen bakteri *E. coli* BW25113 untuk menghasilkan mutan yang tidak memiliki gen penyandi enzim Lon, ClpP, DegP dan OmpT. Melalui teknik perusakan kromosom yang dilanjutkan dengan transduksi menggunakan bakteriophage P1 (*one step chromosomal disruption-P1 phage transduction method*), Ali et al. (2005c) telah berhasil memperoleh mutan di atas, baik mutan tunggal (Δ*lon*, Δ*clpP*, Δ*degP dan* Δ*ompT*) maupun ganda (Δ*degP-ompT*, Δ*degP-lon*, Δ*lon-ompT dan* Δ*clpP-ompT*).

Mutan di atas dipakai untuk membuat ekstrak yang selanjutnya digunakan dalam produksi salah satu protein fungsional yaitu antibodi anti-6D9 dan scFv dari *anti-human serum albumin* (anti-HSA) pada ekspresi dengan sistem transkripsi dan translasi *in vitro*. Hasil pengujian dengan *sodium-dodesyl sulfate polyacrylamide gel electrophoresis* (SDS-PAGE) yang dilanjutkan dengan autoradiografi menunjukkan bahwa penggunaan ekstrak mutan ganda menghasilkan kedua antibodi di atas dalam jumlah yang jauh lebih banyak dibandingkan dengan jumlah antibodi yang dihasilkan dengan penggunaan ekstrak mutan tunggal maupun tanpa mutan (*wild type*) (Ali et al. 2005a). Selain itu, hasil uji dengan *enzyme-linked immunosorbent assay* (ELISA) menunjukkan bahwa penggunaan ekstrak mutan ganda menghasilkan antibodi yang mempunyai aktivitas lebih tinggi dalam berikatan dengan antigen dibandingkan dengan yang dihasilkan dengan ekstrak mutan tunggal maupun tanpa mutan (Ali et al. 2005a).

Penggunaan ekstrak mutan ganda tersebut, terutama mutan *degP-ompT*, mampu menghasilkan antibodi sebanyak yang dihasilkan oleh sistem hibridoma yaitu sekitar 20 µg/ml. Untuk itu, penggunaan mutan tersebut sebagai sumber ekstrak sangat bermanfaat baik untuk produksi berbagai macam protein fungsional, vaksin, antibodi maupun untuk tujuan skrining terhadap pustaka gen penyandi protein fungsional tertentu yang memerlukan waktu cepat (Ali et al. 2005a).

Berhubung manfaat dari mutan baru yang dihasilkan tersebut dalam produksi antibodi menggunakan *cell-free*, maka mutan ganda tersebut sampai saat ini terus dipakai sebagai sumber ekstrak untuk membuat berbagai macam antigen, antibodi, enzim dan protein fungsional lainnya (Ali 2006).

PROSPEK TEKNOLOGI TRANSKRIPSI DAN TRANSLASI *IN VITRO* UNTUK MENGHASILKAN PROTEIN FUNGSIONAL LAINNYA

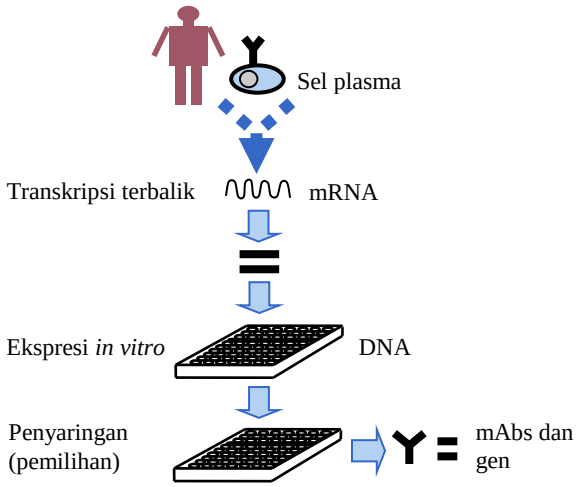
Teknik ini tidak hanya dipergunakan untuk menghasilkan vaksin (Bommakanti et al. 2010; Lu et al. 2014), tetapi juga dipergunakan untuk berbagai keperluan seperti mempelajari sistem *folding* pada sintesis enzim, hormon, protein transpoter dan jenis protein lainnya (Yang et al. 2000), produksi antibodi monoklonal (Ali et al. 2005a; 2006) atau rekayasa antibodi (Nakano & Yamane 2004). Aplikasi ini diiringi dengan upaya perbaikan, baik terhadap ekstrak bakteri yang digunakan (Ali et al. 2005a), sumber energi, jenis bioreaktor maupun aplikasinya dengan teknologi lain untuk meningkatkan penggunaan teknologi transkripsi dan translasi *in vitro* (Ali et al. 2006).

Seiring dengan ditemukannya urutan DNA aneka jenis gen dari berbagai makhluk hidup saat ini, penentuan fungsi dan peranan gen tersebut beserta jenis protein yang dihasilkan dapat diketahui secara cepat dengan menggunakan sistem ekspresi transkripsi dan translasi *in vitro* (Sabrina et al. 2010).

Antibodi monoklonal merupakan protein fungsional yang paling banyak dibuat dengan menggunakan teknologi transkripsi dan translasi *in vitro*. Sampai saat ini, teknologi produksi antibodi monoklonal hanya mampu menghasilkan antibodi mencit. Hal ini disebabkan karena *myeloma* yang diperoleh dari mencit hanya mampu menyatu (fusi) dengan sel limposit mencit. Walaupun penggunaan teknologi *grafting* dan *humanization* telah memungkinkan penggunaan antibodi tersebut untuk manusia, namun kedua teknologi tersebut sangat rumit dan pelaksanaannya membutuhkan biaya besar. Selain itu, antibodi yang dihasilkan masih memberikan efek samping negatif terhadap manusia, walaupun efek samping tersebut masih dapat ditoleransi.

Skrining sel penghasil antibodi monoklonal baik pada hewan maupun manusia dapat dilakukan melalui isolasi materi genetik penyandi antibodi pada sel tersebut (Wang & Stollar 2000) untuk kemudian dideteksi fungsinya dengan teknologi transkripsi dan translasi *in vitro* (Ali et al. 2006). Untuk memperoleh materi genetik dari sel, diperlukan teknologi lain yaitu teknologi *reverse transcription polymerase chain reaction* (RT-PCR) guna mengkonversi *messenger* RNA (mRNA) penyandi antibodi yang terdapat pada sel penghasil antibodi tersebut menjadi cDNA yang lebih stabil untuk diekspresikan dengan teknologi transkripsi dan translasi *in vitro*.

Teknologi transkripsi dan translasi *in vitro* dan RT-PCR sel tunggal akan membuka peluang untuk menghasilkan antibodi monoklonal untuk manusia. Berhasilnya produksi dan konstruksi pustaka gen penyandi antibodi monoklonal hepatitis B dengan menggunakan sel limposit mencit yang telah diimunisasi sebagai *template* (Ali et al. 2005a; Sabrina et al. 2010), telah memberikan harapan besar untuk menghasilkan antibodi monoklonal terhadap hepatitis B. Melalui teknologi tersebut, pustaka gen antibodi untuk menghasilkan antibodi bagi seorang pasien dapat dibuat dengan cepat melalui sintesis cDNA penyandi antibodi dengan menggunakan sel *lymposite* pasien sebagai *template*. Gambar 3 menunjukkan tahapan yang dapat dipergunakan dalam RT-PCR sel tunggal dan sistem ekspresi *cell-free* untuk menghasilkan antibodi monoklonal (mAb) manusia dengan menggunakan sel plasma pasien sebagai *template*.



Gambar 3. RT-PCR sel tunggal dan teknologi transkripsi dan translasi *in vitro* untuk menghasilkan antibodi monoclonal

Sumber: Ali et al. (2006)

Teknologi transkripsi dan translasi *in vitro* telah memberikan kontribusi yang sangat besar terhadap perkembangan bioteknologi molekuler. Klammt et al. (2008) telah menggunakan teknologi tersebut untuk mempelajari struktur protein membran integral. Protein ini sangat berperan baik untuk mengangkut produk metabolit ke luar masuk sel, menghasilkan energi sel maupun untuk komunikasi antar sel serta antar sel dengan lingkungannya tersebut yang sangat sulit untuk diproduksi secara *in vivo*. Melalui penggunaan teknologi ini, Klammt et al. (2008) telah berhasil membuat, mempelajari struktur dan fungsi beberapa protein membran integral yang sangat penting bagi dunia farmasi saat ini.

Produksi enzim dan beberapa reseptor dan *transporter* dengan menggunakan teknologi transkripsi dan translasi *in vitro* telah dilakukan oleh Betton & Miot (2008). Semua protein fungsional yang sangat sulit disintesis secara *in vivo*, dapat dibuat secara *in vitro* dalam jumlah yang memadai. Lebih lanjut, disimpulkan bahwa penggunaan teknologi tersebut memungkinkan untuk dilakukan studi biokimia terhadap protein-protein fungsional.

KESIMPULAN

Teknologi transkripsi dan translasi *in vitro* merupakan salah satu cara yang paling cepat untuk menghasilkan berbagai vaksin seiring dengan munculnya patogen baru. Teknik ini bahkan mampu menghasilkan protein 1-10 mg per mililiter lebih tinggi jika dibandingkan dengan teknik konvensional yang hanya menghasilkan satu dosis per butir telur. Selain itu, teknologi ini dapat dipergunakan untuk mencari vaksin baru melalui ekspresi gen tertentu dari genom patogen, sehingga sangat bermanfaat dalam skrining vaksin guna mengantisipasi munculnya jenis penyakit baru (*emerging infectious disease*).

UCAPAN TERIMA KASIH

Ucapan terima kasih disampaikan kepada Prof Hideo Nakano dan Asisten Prof. Takaki Kojima (*Laboratory of Molecular Biotechnology, Graduate School of Agricultural Sciences*, Nagoya University, Japan) atas kesempatan yang diberikan kepada penulis sebagai *visiting researcher* untuk memperdalam teknologi transkripsi dan translasi *in vitro*. Juga kepada DIKTI, Kementerian RISTEK DIKTI Republik Indonesia yang telah membiayai bagian dari penelitian ini melalui skim penelitian unggulan strategis nasional (PUSNAS) tahun anggaran 2014-2015.

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WARTAZOA Vol. 25 No. 4 Th. 2015 Hlm. 189-196 DOI: http://dx.doi.org/10.14334/wartazoa.v25i4.1229

Penggunaan Kapang Karotenogenik *Neurospora* dalam Fermentasi Limbah Pertanian untuk Pakan Ternak Unggas

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(Diterima 20 Januari 2015 – Direvisi 28 September 2015 – Disetujui 23 Oktober 2015)

ABSTRAK

Limbah pertanian merupakan biomassa yang berpotensi menggantikan sebagian bahan pakan konvensional, tetapi terdapat kendala dalam pemanfaatannya antara lain serat kasar yang tinggi, serta protein dan karoten yang rendah. Salah satu upaya untuk memperbaiki kualitas bahan pakan tersebut adalah dengan fermentasi menggunakan kapang karotenogenik *Neurospora*. Kapang ini mudah tumbuh pada substrat yang difermentasi secara *aerob* dalam waktu yang singkat. Fermentasi dengan kapang ini mampu menurunkan serat kasar, serta meningkatkan protein kasar dan karoten substrat. Produk fermentasi kapang karotenogenik *Neurospora* dapat dimanfaatkan sebagai pakan unggas untuk mencapai produktivitas dan kualitas produk dengan biaya yang lebih efisien.

Kata kunci: Pakan, fermentasi, karoten, *Neurospora*

ABSTRACT

Use of Carotenogenic *Neurospora* in Fermentation of Agricultural Byproduct for Poultry Feed

Agricultural byproduct is biomass that potential to partly subtitute the conventional feed. However, there are some constraints such as high fiber, low protein and carotene contents. One of the efforts to improve the nutritive value of agricultural byproduct is fermentation using carotenogenic *Neurospora*. This fungi easily and readily grows on substrate fermented in aerobic condition. *Neurospora* fermentation is able to reduce crude fiber, to increase crude protein and carotene content of substrate. Utilization of *Neurospora* fermented product as poultry feed ingredients increased productivity and product quality more efficiently.

Key words: Feed, fermentation, carotene, *Neurospora*

PENDAHULUAN

Limbah pertanian merupakan biomassa yang berpotensi untuk digunakan sebagai pakan unggas karena jumlahnya melimpah. Limbah pertanian yang digunakan adalah bagian yang masih mengandung cukup nutrisi untuk menggantikan sebagian bahan pakan konvensional. Penggunaan bahan pakan alternatif tersebut masih memiliki kendala, karena limbah pertanian memiliki karakteristik yang beragam, kualitas nutrisi seperti protein kasar dan karoten yang rendah dan memiliki kandungan serat kasar tinggi (Pasaribu 2007). Limbah pertanian tanpa proses yang langsung digunakan sebagai bahan pakan alternatif akan memberikan respon negatif karena unggas memiliki keterbatasan dalam mencerna serat kasar. Asupan nutrisi yang kurang dapat menurunkan produktivitas unggas, termasuk asupan karotenoid yang kurang juga akan berpengaruh terhadap penurunan kualitas produk unggas (Fenita et al. 2010a).

Karotenoid yang diperoleh dari ransum berupa hijauan alami ataupun sintetis berpengaruh terhadap kualitas produk unggas, karena unggas tidak dapat memproduksi karotenoid sendiri. Penggunaan pigmen karotenoid sintetis dalam ransum berpotensi meningkatkan biaya pakan karena merupakan bahan impor dan harganya mahal. Karotenoid berfungsi untuk memberikan pigmen warna kulit dan sebagai antioksidan yang aktivitasnya dapat menghambat peroksida lipida (Lee et al. 2010). Karotenoid pada ayam pedaging membuat warna daging lebih terang dan cerah, sehingga lebih dipilih dan disukai konsumen (Prayitno et al. 2010). β-karoten berfungsi sebagai antioksidan yang mencegah terjadi radikal bebas dalam tubuh (Mueller & Boehm 2011). Preferensi konsumen dalam memilih telur adalah yang mempunyai warna kuning kemerah-merahan dan tidak pucat. Misalnya pada telur itik, sebagian besar diolah menjadi telur asin, dimana telur asin yang diolah dari telur itik dengan kuning telur yang pucat akan terlihat tidak menarik dengan rasa yang kurang masir. Warna kuning telur pucat dikarenakan kekurangan pigmen karotenoid (xantofil) (Bortolotti et al. 2003). Berdasarkan penelitian Fenita et al. (2010a) senyawa karotenoid dalam pakan dapat meningkatkan warna, kandungan β-karoten dalam kuning telur dan menurunkan kolesterol.

Produk unggas berupa daging dan telur saat ini menjadi favorit untuk dikonsumsi dan semakin digemari oleh masyarakat, karena mengandung gizi yang dibutuhkan oleh tubuh manusia. Menurut Anton et al. (2005) telur mempunyai kandungan nutrisi yang dibutuhkan untuk tubuh manusia berupa kandungan asam amino, vitamin, mineral dan asam lemak. Daging unggas memiliki kelebihan yaitu mempunyai kandungan protein tinggi, asam amino yang lengkap, lemak jenuh dan kolesterol yang rendah sehingga tidak berpotensi menimbulkan penyakit degeneratif jika dibandingkan dengan daging merah (Boni et al. 2010).

Pengolahan limbah pertanian untuk menjadi bahan pakan harus dilakukan agar dapat meningkatkan kualitas produk limbah pertanian tersebut. Salah satu pengolahan pakan secara biologis adalah dengan fermentasi menggunakan kapang karotenogenik *Neurospora*. Karakter limbah pertanian yang disusun dari kerangka karbon yang berbentuk selulosa dan hemiselusosa memiliki kandungan karbon dan nitrogen serta mineral yang cukup untuk pertumbuhan kapang *Neurospora*. Fermentasi bertujuan untuk meningkatkan kandungan protein kasar, menurunkan serat kasar substrat, namun dengan menggunakan kapang karotenogenik *Neurospora* memberikan keuntungan lain yaitu meningkatnya kandungan karoten yang dihasilkan dari konidia yang berwarna jingga. Dengan peningkatan nilai nutrisi substrat, unggas dapat mencerna produk fermentasi lebih baik. Keuntungan fermentasi oleh kapang karotenogenik *Neurospora* jika dibandingkan dengan sumber dari tanaman untuk menghasilkan β-karoten adalah lebih efisien dari segi waktu, tempat dan biaya karena tidak membutuhkan waktu penanaman tumbuhan, peralatan yang besar dan berat serta tempat yang luas (Novianti et al. 2004). Pemanfaatan limbah pertanian yang difermentasi dengan kapang karotenogenik *Neurospora* sebagai upaya meningkatkan nilai gizi pakan dapat menjadi pakan alternatif untuk meningkatkan produktivitas dan kualitas produk unggas dengan biaya yang lebih efisien (Guntoro & Yasa 2005).

LIMBAH PERTANIAN SEBAGAI PAKAN UNGGAS

Upaya untuk mengetahui potensi sumber bahan pakan baru telah dilakukan dengan mencari sumber pakan alternatif yang ada di Indonesia dengan pertimbangan produktivitas, kontinuitas, jarak dan nutrisi. Berbagai penelitian telah banyak dilakukan untuk mengetahui potensi bahan pakan dengan analisis kandungan nutrisi bahan dan pemberiannya pada unggas untuk mengetahui respon produksi dan seberapa besar proporsi bahan pakan untuk menyusun ransum (Tangendjaja 2007). Bahan pakan asli Indonesia yang berasal dari limbah pertanian misalnya dedak padi, dedak jagung, sagu, limbah kelapa sawit, ampas tahu dan onggok merupakan bahan pakan yang biasa digunakan sebagai ransum unggas (Zainuddin 2005).

Proporsi pemakaian bahan pakan lokal dari limbah pertanian bervariasi (Tabel 1) yaitu antara 10-30%. Faktor yang berpengaruh terhadap jumlah pemakaian bahan pakan adalah nutrisi yang tersedia di dalamnya diantaranya adalah energi metabolisme, protein kasar dan serat kasar (Suprijatna et al. 2012). Kandungan serat kasar yang tinggi pada limbah pertanian sebagai pakan unggas dapat mengurangi palatabilitas dan bersifat *bulky* atau saluran pencernaan terasa penuh, menyebabkan unggas menjadi cepat kenyang akibat konsumsi serat pakan sedangkan di sisi lain konsumsi ransum terbatas mengakibatkan defisiensi nutrisi sehingga dapat menghambat pertumbuhan unggas. Faktor pembatas lain penggunaan sumber pakan alternatif yaitu beberapa bahan pakan mempunyai kandungan senyawa antinutrisi. Tingkat konsumsi dan palatabilitas ternak terhadap suatu bahan pakan alternatif juga perlu diperhatikan, sehingga profil dan karakter nutrien perlu diketahui (Prawirodigdo 2005). Mengacu pada faktor tersebut, bahan pakan alternatif membutuhkan teknologi pengolahan lebih lanjut untuk meningkatkan kualitas nutrisi khususnya penurunan kandungan serat kasar.

Tabel 1. Kandungan nutrisi beberapa jenis limbah pertanian

Bahan pakan	Energi metabolis (kkal/kg)	Protein kasar (%)	Serat kasar (%)	Batas penggunaan dalam ransum unggas (%)
Dedak padi[1)]	3080	11,87	9,97	30
Onggok[2)]	3000-3500[7)]	2,20	16,00	20
Ampas tahu[3)]	2907	22,64	22,65	10
Lumpur sawit[4)]	1125-1593	9,6-14,52	11,50-32,90	20
Ampas sagu[5)]	3508-3860	0,92-1,01	9,22-10,50	10[8)]
Bungkil kelapa[6)]	1667	18,60-25,00	12,00-14,44	20[9)]

Sumber: [1)]Hartadi et al. (1997); [2)]Mulyono et al. (2011); [3)]Tanwiriah et al. (2006); [4)]Sinurat (2003); [5)]Uhi (2007); [6)]Sinurat et al. (1995); [7)]Yohanista et al. (2014); [8)]Ulfah & Bamualim (2002); [9)]Mathius & Sinurat (2001)

Pada umumnya, masih sedikit penelitian bahan pakan lokal yang menyajikan data kandungan karotenoid dalam bahan pakan. Mengingat urgensi pertumbuhan konsumen yang memilih produk berdasarkan kualitas mengalami peningkatan, bahan pakan lokal yang berkualitas perlu diketahui kandungan karotenoidnya agar dapat menghasilkan produk unggas yang berkualitas. Salah satu cara meningkatkan kandungan karotenoid bahan pakan adalah fermentasi dengan kapang karotenogenik *Neurospora*.

KAPANG KAROTENOGENIK *NEUROSPORA*

Kapang karotenogenik yang dapat menghasilkan pigmen karotenoid berasal dari genus *Neurospora* dari golongan *heterothallic* dan *pseudomothallic*. Karakteristik dari golongan ini adalah makro dan mikro konidianya berwarna kuning hingga jingga karena mengandung senyawa karoten, sedangkan pada konidia dari golongan *homothallic* tidak mengandung senyawa karoten sehingga mempunyai warna cokelat hingga hijau (Perkins & Turner 1988). Karotenoid adalah sebuah golongan senyawa dengan rantai karbon panjang (C40) dan terdiri dari bermacam-macam jenis (>600 molekul) yang disintesis oleh tanaman. Hewan tidak dapat membuat sendiri karotenoid dalam tubuhnya, sehingga harus memperolehnya dari ransum yang mengandung karotenoid. Karotenoid biasanya memberikan warna kuning, jingga dan merah. Karotenoid terbagi dalam dua golongan besar yaitu golongan karoten dan golongan xanthofil (Wina 2008). Kapang karotenogenik *Neurospora* telah banyak digunakan untuk pembuatan makanan tradisional di Indonesia, misalnya pembuatan oncom melalui proses fermentasi sehingga dikenal dengan nama ragi oncom. Karotenoid yang diproduksi kapang karotenogenik *Neurospora* didominasi oleh β-karoten, di atas 50% dari total karotenoid yang ada (Kenyamu et al. 2014).

Klasifikasi ilmiah untuk *Neurospora* adalah genus *Neurospora*, familia *Sordariaceae*, ordo *Sordariales*, *class Ascomycetes*, filum *Ascomycota* dan *kingdom* fungi. *Neurospora* mempunyai ciri hidup berkoloni dengan warna kuning sampai *orange* pucat. Organisme ini ditemukan pada tanah dan sisa-sisa vegetasi (García et al. 2004). Lima spesies dari *Neurospora* yang dikenal sebagai penghasil karotenoid yaitu dari *heterothallic* dengan spesies *Neurospora crassa*, *Neurospora discreta*, *Neurospora intermedia*, *Neurospora sitophila* dan dari *pseudomothallic* dengan spesies *Neurospora tetrasperma* (Perkins & Turner 1988; Jacobson et al. 2006).

LINGKUNGAN DAN SIKLUS HIDUP *NEUROSPORA*

Kehidupan dan pertumbuhan kapang dipengaruhi oleh kondisi lingkungan substrat yang digunakan. Substrat merupakan sumber nutrisi utama bagi fungi agar kapang mengekskresikan enzim ekstra seluler yang dapat mengurai senyawa kompleks dari substrat tersebut menjadi senyawa yang lebih sederhana. Suhu ruangan untuk tumbuh kapang yaitu pada 25-30°C dengan kelembaban 70-90% (Kalsum & Sjofjan 2008). pH substrat yang optimum untuk pertumbuhan kapang *Neurospora* adalah 5,5 dimana aktivitas mikroorganisme optimum sehingga memproduksi enzim yang dapat mengurai substrat (Syahruddin et al. 2011).

Kapang karotenogenik *Neurospora* dapat mudah memperbanyak konidia jingga sebagai sumber karotenoid dengan cepat dan banyak karena reproduksi dilakukan dengan dua cara, yaitu secara seksual dan aseksual (Fleißner et al. 2008). Reproduksi aseksual pada *Neurospora* dilakukan dengan cara membentuk tunas (*budding*), konidia dan fragmentasi. Tunas yang telah masak akan terlepas dari sel induknya dan tumbuh menjadi individu baru. Konidia adalah spora yang dihasilkan dari diferensiasi dengan membentuk sekat melintang pada ujung hifa hingga terbentuk banyak konidia, ketika telah masak konidia paling ujung akan melepaskan diri (Tan 2003). Reproduksi seksual terjadi dengan cara membentuk askospora (spora seksual yang terbentuk di dalam askus). Askospora terbentuk melalui hifa (+) membentuk alat kelamin jantan (anteredium) dan hifa (-) membentuk alat kelamin betina (askogonium) yang bertemu dan terjadi plasmogami (penyatuan sitoplasma) tanpa disertai penyatuan inti. Jadi, dalam peristiwa tersebut akan terbentuk sel dengan dua inti askogonium yang telah memiliki dua inti tersebut akan menghasilkan hifa-hifa askogonium yang dikariotika (berinti dua). Hifa dikariotika itu bercabang-cabang membentuk tubuh buah yang disebut askokarp. Ujung hifa dikariotika akan membentuk sel khusus yang akan menjadi askus. Di dalam askus akan terjadi perleburan dua inti. Selanjutnya, inti askus membelah dua kali. Pembelahan pertama terjadi secara meiosis dan menghasilkan empat sel. Pembelahan kedua terjadi secara mitosis sehingga terbentuk delapan aksopora di dalam askus tersebut (Radic 2011).

PEMBUATAN FERMENTASI PAKAN DENGAN KAPANG KAROTENOGENIK *NEUROSPORA*

Fermentasi pada prinsipnya menyediakan media yang mempunyai nutrisi untuk pertumbuhan mikroorganisme. Media untuk pertumbuhan kapang karotenogenik *Neurospora* membutuhkan substrat padat yang memiliki kandungan sumber karbon dan nitrogen dan mineral sebagai nutrien. Media fermentasi dengan kandungan nutrien yang seimbang diperlukan untuk menunjang kapang lebih maksimal dalam memproduksi β-karoten (Fenita et al. 2010a). Kapang karotenogenik *Neurospora* mudah tumbuh pada substrat secara aerob, mempunyai waktu generasi yang pendek dan miseliumnya terdiri dari hifa yang bercabang, menjulang ke udara, yang mudah dikenal dari konidianya yang berwarna jingga.

Nutrisi lain yang penting bagi pertumbuhan kapang karotenogenik *Neurospora* salah satunya adalah mineral. Mineral dibutuhkan untuk pertumbuhan kapang, produksi enzim dan dapat memperbaiki produksi karoten (Priatni 2014). Kebutuhan mineral disesuaikan dengan kandungan unsur di dalam substrat dan sel kapang tersebut sehingga pada limbah pertanian yang akan digunakan sebagai substrat apabila kandungan mineralnya rendah dapat dilakukan penambahan mineral. Mineral seperti Fe, Cu, Co, Zn, Mg, Mo dan Cl dibutuhkan untuk mendukung kerja fungsi sel kapang (Ikatrinasari 1995). Berdasarkan penelitian Noverina et al. (2013) suplementasi sulfur dengan nitrogen memberikan interaksi terhadap kandungan protein kasar, protein murni, lemak kasar, serat kasar pada tongkol jagung hasil bioproses kapang *Neurospora sitophila*. Larutan mineral standar (Vogel) yang digunakan dalam media fermentasi *Neurospora crassa* adalah dalam bentuk larutan NH_4NO_3, $MgSO_4.7H_2O$, KH_2PO_4, $CaCl_2$ (Dogaris et al. 2009).

Fermentasi pakan dengan kapang karotenogenik *Neurospora* diawali dengan menyiapkan substrat ditambah aquades (kadar air 70%) dan mineral yang ditimbang berdasarkan kebutuhan untuk kemudian diaduk secara merata. Proses berikutnya adalah sterilisasi dengan cara pengukusan substrat selama 30-45 menit setelah air mendidih, setelah itu substrat didinginkan dengan cara dibiarkan sampai tercapai suhu kamar. Substrat kemudian diinokulasi dengan inokulum kapang karotenogenik *Neurospora* sebanyak 9% dan diaduk secara merata. Substrat yang telah diinokulasi dengan kapang karotenogenik *Neurospora* kemudian diinkubasi secara aerob selama tujuh hari. Setelah itu, produk fermentasi dipanen, dikeringkan dioven dan digiling (Nuraini et al. 2008; Fenita et al. 2010b).

FERMENTASI PAKAN DENGAN KAPANG KAROTENOGENIK *NEUROSPORA* PADA BERBAGAI SUBSTRAT

Selama fermentasi kapang karotenogenik *Neurospora* dengan substrat, terjadi perubahan kandungan nutrisi substrat yang bervariasi (Tabel 2), akibat adanya aktivitas enzim yang dihasilkan oleh kapang tersebut. Hasil dari produk fermentasi ditentukan oleh enzim yang diproduksi, mikroorganisme yang tumbuh dan kandungan bahan. Penelitian yang dilakukan oleh Li et al. (2014) ketika melakukan isolasi *Neurorospora crassa* dan menganalisis enzim yang diproduksi menemukan adanya aktivitas enzim peptidase (protease), endoglukanase, eksoglukanase, β-glukosidase, *cellobiose dehydrogenase*. Enzim *cellobiose dehydrogenase* merupakan enzim ekstraseluler yang berperan dalam hidrolisis selulosa dan hemiselulosa. Fermentasi menggunakan *Neurospora* yang memiliki sifat selulolitik mampu memecah ikatan selulosa sehingga menyebabkan kandungan serat kasar dalam substrat turun. Serat kasar sebagai pembatas bagi ternak unggas karena unggas tidak menghasilkan enzim selulase (Martaguri et al. 2011). Fermentasi menggunakan *Neurospora* menyebabkan degradasi selulosa, hemiselulosa dan polimernya menjadi gula sederhana atau turunannya serta mampu meningkatkan nutrisi bahan substrat (Mahfudz 2006a). Enzim selulase terdiri dari endo-β-1,4-glukanase dan ekso-1,4-glukanase dan β-glukosidase yang bekerja secara sinergis (Dogaris et al. 2009). Endo-β-1,4-glukanase memotong ikatan rantai dalam selulosa menghasilkan molekul-molekul selulosa yang lebih pendek. Ekso-1,4-glukanase memotong ujung rantai selulosa menghasilkan molekul selobiosa, sedangkan β-glukosidase memotong molekul selobiosa menjadi dua molekul glukosa (Romero et al. 1999).

Peningkatan β-karoten terjadi karena kapang karotenogenik *Neurospora* dapat mensintesis *geranyl geranyl diphosphate* (GGDP) hingga akhirnya menjadi β-karoten (Díaz-Sánchez et al. 2011). Faktor yang berpengaruh terhadap biosintesis karoten adalah kondisi medium, level dan aktivitas enzim yang mensintesis karoten, cahaya, suhu, kandungan kimia dan mineral (Priatni 2014). Biosintesis β-karoten dimulai dari molekul GGPP memproduksi karoten-phytoene yang dikatalisis oleh enzim al-2. Pada awalnya, hingga lima ikatan ganda terkonjugasi menjadi phytoene dimediasi oleh al-1, sehingga terbentuk 3,4-didehydrolycopene yang dimulai dari terbentuknya phytofluene, ζ-karoten, likopen dan *neurosporene*. Selanjutnya akan menjadi β-karoten dengan bantuan enzim al-2 (Díaz-Sánchez et al. 2011).

Tabel 2. Fermentasi kapang karotenogenik dengan berbagai substrat

Mikroorganisme	Substrat	Perubahan nutrisi produk setelah dilakukan fermentasi		Sumber
		Sebelum	Sesudah	
Neurospora sp	Limbah cair tahu, air kelapa, onggok ditambah mineral NH_4NO_3 0,15%; $MgSO_4$.7H2O 0,1%; KH_2PO_4 0,25%	t.a	Dihasilkan karoten tertinggi yaitu 108,26 µg/100 ml	Nuraida et al. (1996)
Neurospora sitophila	Campuran ampas tahu dan onggok	PK 16,46%; LK 4,25%; HCN 0,43%; antitripsin 5,97%	PK 23,94%; LK 6,57%; HCN 0,38%; antitripsin 5,51%	Kalsum & Sjofjan (2008)
Neurospora crassa	Campuran onggok dan ampas tahu	Lisin 0,23%; metionin 0,10%; triptofan 0,05%	Lisin 1,58%; metionin 0,37%; triptofan 0,13%	Nuraini et al. (2008)
Neurospora crassa	Kombinasi onggok, ampas tahu, bungkil sawit, bekatul	t.a	*Level* karbon:nitrogen = 60:40%, diperoleh β-karoten 234,32 µg/g; PK 15,63% dan SK 13,10%	Nuraini et al. (2009)
Neurospora sp	Lumpur sawit ditambah mineral 3,6% (NH_42SO_4; NaH_2PO_4 0,75%; $MgSO_4$ 0,25%; KCl 0,075%)	PK 13,57%; total asam amino 7,02%; lisin 0,34%; metionin 0,29%; β-karoten 1873,40 µ/100g; SK 28,03%	PK 23,45%; total asam amino 8,54%; lisin 0,39%; metionin 0,36%; β-karoten 3735,80 µ/100g; SK 23,45%	Fenita et al. (2010a)
Neurospora sp	Ampas sagu	PK 3,29%; SK 18,5%	PK 13,15%; SK 14,28%	Martaguri et al. (2011)
Neurospora sitophila	Tepung daun mengkudu	β-karoten dalam ransum 14,80 mg/kg	β-karoten dalam ransum 118,40 mg/kg	Syahruddin et al. (2011)

t.a: Tidak ada data

Golongan karoten dikenal sebagai prekusor provitamin A. Fenita et al. (2010a) melaporkan terjadi peningkatan kandungan β-karoten pada substrat jika dibandingkan dengan sebelum difermentasi dengan kapang *Neurospora* sp.

Fermentasi dengan kapang *Neurospora* memiliki kemampuan menguraikan protein beserta asam amino (Banerjee et al. 1995). Fermentasi dengan *Neurospora* dapat memproduksi enzim protease yang dapat mencerna protein menjadi asam amino dan lipase yang mencerna lemak, trigliserida menjadi asam lemak bebas (Kurniati 2012).

PENGGUNAAN BAHAN PAKAN YANG DIFERMENTASI DENGAN KAPANG KAROTENOGENIK *NEUROSPORA* PADA TERNAK UNGGAS

Produk fermentasi dengan menggunakan kapang karotenogenik *Neurospora*, dapat dikonsumsi dan memberikan pengaruh yang baik terhadap produktivitas unggas pedaging dan petelur (Tabel 3). Berdasarkan hasil penelitian (Mahfudz 2006a) diperoleh hasil terbaik penggunaan produk fermentasi dengan level 15%, itik mampu memanfaatkan protein dengan baik, sehingga dapat menggantikan sebagian bahan pakan sumber protein. Pemberian pakan fermentasi dengan kapang karotenogenik *Neurospora* dengan level tertinggi (20%) pada ayam *broiler* memiliki efek positif terhadap produktivitas meliputi peningkatan bobot badan dan persentase karkas pada ayam *broiler* sehingga memberikan *income over feed cost* (IOFC) yang besar terhadap keuntungan peternak (Mahfudz 2006b). Baik pada unggas pedaging maupun petelur, hasil yang tidak berbeda nyata dengan kontrol akibat dari penggunaan produk karotenogenik *Neurospora*, membuktikan tidak terdapat pengaruh negatif (penurunan produktivitas) dari penggunaan produk (Fenita et al. 2010b; Nuraini et al. 2014; Desi et al. 2015). Uraian tersebut membuktikan produk fermentasi dengan kapang karotenogenik *Neurospora* mempunyai nilai nutrisi yang baik serta dapat dimanfaatkan sebagai sumber nutrisi unggas dengan baik yang ditandai dengan produktivitas yang sama dengan perlakuan kontrol atau meningkat, sehingga dapat digunakan sebagai bahan pakan alternatif untuk menggantikan sebagian bahan pakan konvensional. Penggunaan limbah pertanian yang telah difermentasi dengan kapang karotenogenik *Neurospora* mengakibatkan jumlah proporsi limbah pertanian yang digunakan dalam menyusun ransum meningkat jika dibandingkan dengan limbah pertanian tanpa proses fermentasi. Pemberian produk fermentasi dengan kapang karotenogenik *Neurospora* memberikan efek terhadap kualitas daging dan telur (Tabel 4). Penurunan kandungan kolesterol daging terjadi pada ayam *broiler* yang diberikan pakan fermentasi dengan kapang karotenogenik *Neurospora* (Syahruddin et al. 2011).

Tabel 3. Penggunaan pakan yang difermentasi dengan kapang karotenogenik dan efeknya terhadap produktivitas unggas

Mikroorganisme	Substrat	Proporsi dalam ransum perlakuan	Respon pada ternak	Sumber
Neurospora sitophila	Ampas tahu	7,5-15%	Pemberian fermentasi ampas tahu *level* 15% tidak berpengaruh nyata terhadap efisiensi penggunaan protein pada itik	Mahfudz (2006a)
Neurospora sp	Ampas tahu	10-20%	Terjadi peningkatan peformans (konsumsi, efisiensi ransum, PBB) dan bobot karkas pada *broiler*	Mahfudz (2006b)
Neurospora sp	Lumpur sawit	15%	Penggunaan lumpur sawit dalam ransum tidak berpengaruh nyata terhadap konsumsi, konversi, produksi telur dan IOFC	Fenita et al. (2010b)
Neurospora crassa	Tepung kulit pisang dan ampas tahu	0-20%	Tidak berpengaruh nyata terhadap konsumsi, PBB, bobot badan, konversi ransum dan persentase karkas	Nuraini et al. (2014)
Neurospora crassa	Tepung kulit pisang	3-21%	Pemberian tepung kulit pisang tidak berpengaruh nyata terhadap bobot karkas dan persentase karkas	Desi et al. (2015)

PBB: Pertambahan bobot badan

Tabel 4. Pengaruh pemanfaatan pakan fermentasi kapang karotenogenik terhadap kualitas produk unggas

Jenis substrat	Penggunaan substrat dalam ransum (%)	Kolesterol produk (mg/100 g)	Karoten telur (µg/100 g)	Skor warna kuning telur	Sumber
Campuran onggok dan ampas tahu	0	207,20	td	8,40	Nuraini et al. (2008)
	10	175,40	td	9,00	
	20	143,40	td	10,00	
	30	117,80	td	10,60	
Lumpur sawit	0	309,30	452,30	5,83	Fenita et al. (2010a)
	5	286,40	523,40	7,14	
	10	282,10	645,80	7,97	
	15	253,20	687,90	8,67	
	20	246,00	723,50	9,88	

td: Tidak diamati

Kandungan kolesterol turun dengan semakin tingginya substrat fermentasi dalam ransum, yaitu dari 73,17 mg/100 g (kontrol) menjadi 31,49 mg/100 g saat substrat tepung daun mengkudu di dalam ransum sebesar 21%. Berdasarkan penelitian Nuraini et al. (2008) diperoleh peningkatan bobot telur, warna kuning telur dan kandungan kolesterol yang rendah jika dibandingkan tanpa pemberian produk fermentasi. Kandungan kolesterol pada daging ayam *broiler* turun dari 75,08 mg/100 g (kontrol) menjadi 55,43 mg/100 g ketika campuran asmpas sagu-tahu yang difermentasi *Neuspora* digunakan sekitar 21% di dalam ransum. Pemberian produk fermentasi yang meningkat memiliki hasil yang positif selaras dengan peformans dan kualitas telur. Demikian juga hasil dari penelitian Fenita et al. (2010a), semakin tinggi penggunaan produk fermentasi semakin meningkat pula kualitas telur, karena terjadi peningkatan karoten dan penurunan kolesterol jika dibandingkan dengan kontrol yaitu karoten 452,3 *vs* 723,5 µ/100g dan kolesterol 309,3 *vs* 246 mg/butir. Peningkatan konsumsi β-karoten mengakibatkan penurunan kandungan kolesterol karena β-karoten dapat menghambat kerja enzim HMG-KoA reduktase (hidroksimetil glutaril-KoA) yang berperan dalam pembentukan mevalonat pada proses biosintesis kolesterol (Laszo et al. 2005). Telah terbukti bahwa produk pakan fermentasi dengan kapang karotenogenik *Neurospora* dapat digunakan sebagai bahan pakan penyusun ransum serta meningkatkan kualitas produk unggas.

KESIMPULAN

Limbah pertanian yang difermentasi dengan kapang karotenogenik *Neurospora* dapat digunakan sebagai alternatif bahan pakan bagi ternak unggas dengan memperhatikan proporsi dalam ransum. Fermentasi kapang karotenogenik *Neurospora* dapat meningkatkan nutrisi bahan pakan berupa penurunan serat kasar, peningkatan protein kasar dan karotenoid sehingga unggas dapat mencapai produktivitas dan kualitas produk dengan biaya yang lebih efisien.

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WARTAZOA Vol. 25 No. 4 Th. 2015 Hlm. 197-205 DOI: http://dx.doi.org/10.14334/wartazoa.v25i4.1230

Technology in Feeding Management to Increase Ruminant Productivity

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(Diterima 16 Januari 2015 – Direvisi 21 Agustus 2015 – Disetujui 23 Oktober 2015)

ABSTRACT

Traditional feeding management in ruminants is defined by allowing the animal to find their own feed, consisted of raw materials of grasses and leguminous foliages as much as possible (*ad libitum*) to get a high productivity; however, it needs longer period of time to reach maximum level of production. An advanced feeding management of ruminant is defined as: (1) Processing feed ingredients to improve the nutritive value; (2) Supplementing the animal with substances into the dietary formula to manipulate the rumen ecosystem either by reducing the protozoa population, increasing the concentration of certain nutrients, changing the rumen characteristics; and (3) Changing the site of digestion of the nutrients to increase the absorption and feed utilization. Many research works have been carried out to evaluate the effects of process technology on the efficiency of feed utilization. Improving feeding management will increase livestock production.

Key words: Feeding management, supplement, rumen ecosystem, productivity

ABSTRAK

Teknologi dalam Pemberian Pakan untuk Meningkatkan Produktivitas Ruminansia

Manajemen pemberian pakan ternak ruminansia secara tradisional adalah dengan cara menggembalakan ternak untuk memperoleh hijauan pakan berupa rumput dan leguminosa secara *ad libitum* untuk menghasilkan produksi yang optimal. Cara pemberian pakan tersebut memerlukan waktu lama untuk menghasilkan produksi ternak yang tinggi. Pakan berteknologi maju melalui: (1) Pengolahan bahan pakan untuk meningkatkan kualitas nutrisi bahan tersebut; (2) Pemberian pakan suplemen untuk mempengaruhi kinerja ekosistem rumen dengan menekan populasi protozoa, meningkatkan konsentrasi nutrien tertentu, merubah karakteristik rumen; dan (3) Proteksi bahan pakan dari kecernaan di rumen untuk meningkatkan penyerapan dan pemanfaatan pakan. Telah banyak dilakukan penelitian untuk menguji pengaruh pengolahan bahan pakan terhadap efisiensi pemanfaatan bahan pakan tersebut. Perbaikan manajemen pemberian pakan dapat meningkatkan produktivitas ternak.

Kata kunci: Manajemen pemberian pakan, suplemen, ekosistem rumen, produktivitas

INTRODUCTION

Ruminant animals, in their natural habitat, basically eat fibrous feeds as nutrient resources for their maintenance and production requirements. Natural grasses and leguminous foliages are the primary resources of fibrous feeds. However, ruminant animals will not be able to digest the fibrous nutrients from those grasses and leguminous foliages without the help of microbial enzymatic activities in the digestive tract. The interrelationship between ruminants and the microbial species inhabiting in the rumen plays basic knowledge of the current feeds and feeding management to maximize the efficiency of fibrous feed utilization. Host animals can help the microbial enzymatic activity by eating processes series to increase the surface area of feed particle by reducing the sizes, such as by chewing, mastication, regurgitating, mixing the digested feeds and transporting along the digestive tract. The microbes help hydrolyzing the feed particle by providing enzymes necessary for the digestion of respective nutrients, i.e., protein, lipids and fibrous components. The highly specificity of the enzymes to each chemical reaction makes the microbes to exert many kind of enzymes suited the needs of nutrient components, either for degrading down or building up processes. Therefore, hundreds of microbial species could be found in the rumen ecosystem, with their respective specific interaction and synergetic activities.

Ruminants require all nutrients for the normal productive physiological metabolism. In addition to the major nutrients such as protein, carbohydrates, and lipids, ruminants also need minerals, vitamins and other essential nutrients. In general, the animal requires feed to fulfill the needs for energy, which may come from carbohydrate, lipids and protein, whereas the products of animals may also contain the necessary components such as protein, fat, vitamins and minerals in a variable concentration. Ruminants may use protein from feeds and/or protein from microbial mass cells for their tissue metabolisms. Therefore, increasing the

microbial protein synthesis *de novo* is an alternative strategy to satisfy the protein needs of the animal. The efficiency of microbial protein synthesis depends on the availability of ammonia and energy in the rumen ecosystem, therefore it can be enforced by manipulating the rumen ecosystem. The greatest challenge of tomorrow's nutritionist is to provide local feed energy resources and to enhance nutrient utilization, allowing a high yield of animal product without reliance on imported cereal grains and oilseed meals (Hegarty 2012).

The objective of the present paper is to describe the advanced feeding system in comparison to the common system carried out by farmers with natural raw feedstuffs. Technology for feedstuffs treatments and the appropriate use of the feedstuffs in the total mixed ration is expected to improve the ruminant production.

NATURAL VERSUS HOUSED FEEDING OF LIVESTOCK

Natural feeding versus housed feeding is distinguished by the feedstuffs being given to the animal. In natural feeding, the animal is fed with raw feedstuffs without any processing technique applied and the animal is allowed to choose them freely such as those in a grazing system or in a limited cut and carry cafeteria feeding system. In the housed feeding system, the feedstuffs are either being processed (chemical, physical or biological) before it is offered to the animal, and may be mixed in a formulated ration, or supplemented with any substance which is not taken by the animal under natural feeding condition. Therefore, there are two possible systems, which are known as extensive and intensive feeding systems.

Extensive system

In extensive feeding system, the animals are allowed to find their own needs for energy and other nutrients by scavenging grasses or green leaves within an open grazing land, pasture or under estate crop in an integrated crop-livestock system without intervention from farmers, except fencing from paddock to paddock. The productivity of animals is highly depended on the quality and quantity of grasses and green leaves available in the area.

Extensive feeding practice in grazing management actually is the most efficient feeding systems since there is not significant cost required. However, the production of the livestock is generally low and takes a longer time. Under grazing system, the nutrient availability may be inadequate to fulfill the animal's requirement for higher production due to the lack of some essential nutrients. The nutrient contents of the vegetation in a grazing area depend on the soil fertility and also on the vegetation characteristics. Supplementation with mineral or salts may be required for animals in grazing system.

Intensive system

Contrary to the extensive feeding system, in intensive feeding system the animals are fed with grasses or green leaves, concentrate and other supplements to fulfill the daily energy and nutrient requirements. The feeds may be formulated by farmers using several feed ingredients in a total mixed ration, includes supplements for minerals and vitamins. It is possible to modify the dietary nutritive value by formulating rations using several locally available feedstuffs, which may provide adequate nutrients at a level required for maximal livestock production. The animals are not allowed to find the feed voluntarily but they are forced to receive the offered feeds as a total mixed ration (TMR). In intensive feeding system, the animal depends on the animal raiser (farmer) for their daily needs of feed because they cannot find the feed by themselves. In these circumstances, the farmer should offer the appropriate feed, either in the form of grass forages, leguminous foliages or concentrate feed.

BASIC CONCEPT OF NUTRITION IN RUMINANTS

Basic concept behind the practical feeding management is related to the process of digestion, fermentation and metabolism that should be inter-connected each other so that, there will be a continuous flow of nutrients from mouth to animal products. Feed consumed by the animal will be chewed in the mouth, masticated, mixed with digestive enzymes, transferred to the rumen for further enzymatic digestion and fermentation with the help of microbes in the rumen; before further digestion, the bolus of digesta will be regurgitated and re-masticated to reduce the particle size even further to make it easier for the enzymatic digestion. After ruminal fermentation then the feed particles will be transported to the lower digestive tract for further hydrolysis and absorption through the rumen or intestinal wall following the circulation of the blood to the liver. Nutrients metabolism may take place in the liver or in the tissue level before being deposited as animal products in the form of meat or milk, protein or fat depending on the nutrient balance condition.

Providing nutrients for optimum animal production, i.e., at a level close to that of the genetic potential of the animal can be carried out by fulfilling all the requirements for metabolic needs. For instance,

if the animal can produce daily live weight gain at a rate of 1 kg then all the nutrients deposited in this gain should be satisfied from the metabolized nutrients. Therefore, considering the quantity of nutrient intake, digestion, fermentation, absorption, metabolism and deposition, the feed can be formulated, so that the animal requirement is satisfied. Even though interaction among those factors is important, those factors can be fractionated from one to another for further elucidation.

Nutrient intake is affected by the feedstuff's palatability and carrying capacity of the digestive tract, especially the capacity of rumen space to keep the feed for a certain period of time before being transferred to the subsequent section of the tract.

Digestion is affected by the structural characteristics of the feedstuffs, availability of enzymes and the microbial enzyme variability and suitability to the feed components. The interaction between feedstuffs and the enzymes play an important role in the digestion process. The synergistic effects of the available microbes in producing enzymes necessary for the process of digestion is also an important factor.

Fermentation of feed organic matter is carried out by microbial enzymes with ultimate production of volatile fatty acids, CO_2, H_2, methane and microbial mass. Ruminants rely on volatile fatty acids for the energy requirement. The microbial mass is used by the animals as source of protein. The higher content of lignocellulosic material in the feed generally resulted in higher production of methane, suggesting that more energy from feed is not utilized by the animal. Approximately 15% of the intake of dietary gross energy is loss as methane.

Absorption is affected by the healthiness of the digestive tract where the site of absorption occurs. Most parts of the microbial fermentative results are absorbed in the rumen or abomasum, and some parts will be absorbed along the intestinal tract, especially in the duodenum. The caecum is also an important site of nutrients absorption, especially for the hind gut fermentation.

Metabolism of nutrients which mostly take place in the liver and other tissues determine the ultimate fate of the nutrient intake, whether they will be deposited as protein or fat, either in meat or milk. The metabolic processes are not that simple due to thousands of enzyme catalyzed chemical reactions involve in the whole processes. It is only for simplicity that metabolism is associated with two processes, i.e., anabolism and catabolism depending on the nutrient balances.

The main objective of keeping livestock is to provide animal's products for human consumption. Meat, milk and other derivative products of them are the major target of more efficient livestock management. Since most production cost of livestock is devoted for feed, therefore it is understandable that attention should be directed to how to make efficient feeding expenses.

Role of microbes in feed degradation

Ruminants have specific digestion characteristics due to the activity of microbes in the rumen. The microbes can be classified as bacteria, protozoa and fungi. These microbes have interaction and synergism among each other in utilizing of organic material from feeds. The interaction may be antagonistic, symbiotic, or synergistic. The ecological system of the rumen changes dynamically from time to time depending on the process of organic matter digestion and fermentation. The factors affecting the dynamic ecosystem of rumen includes pH, osmotic pressure, ammonia concentration, CO_2 and H_2 concentration, nutrients availability, volatile fatty acids concentration and may be antibiotic. The enzymatic potency of the rumen microbes is the key factor in dietary nutrient utilization in ruminants. Therefore, the benefit of rumen microbial activity is that the animal will have adequate available nutrients by utilizing the microbial fermentation products as well as the microbial mass itself. Nutrient dynamics, however, impact not only on pathway inputs but also the turnover and output of the whole ecosystem. Knowledge of the optimal balance of metabolic processes and the corresponding microbial taxis required to provide a stable, balanced ecosystem would enable a more holistic understanding of the rumen (Edwards et al. 2008).

MANIPULATING EFFICIENCY OF FEED UTILIZATION

Changes in rumen ecosystem can be done by modulated the activity of rumen microbes in producing the enzymes required for the fermentation of feed organic matter (Haryanto 2014). These dynamic changes of rumen ecosystem open the possibility to manipulate the dietary nutrient utilization. Affecting the hydroxy ion concentration (pH) may change population of the rumen microbes, especially the protozoa and bacteria, by which the synergistic process of the available microbes will drive the pH back to the normal condition; meanwhile the fermentation of feed organic matter resulted in the formation of acids which will reduce the pH, then the population of protozoa will increase and as predator of bacteria, therefore the population of bacteria will be lowering down and followed by increasing back the pH to normal. This process occurs continuously resulting in the dynamic system of rumen fermentation activities. Similarly, the

concentration of ammonia increased the microbial protein synthesis then followed by lowering down the concentration of ammonia in the rumen; the urea cycle in the ruminants help maintaining ammonia concentration in the rumen by gradient diffusion between blood circulatory system and the rumen ecosystem.

Soluble fraction of forage

Changing the degradability of nutrients in the rumen may change the site of digestion, which is expected to be more efficiently utilized by the animal; changing the osmotic pressure, such as saponification may change the microbial population, hence the fermentation process will also change. Supplementation of polyether antibiotics may modify the microbial population and may change the fermentation activity. Supplementation of soluble fraction of forages may also affect the efficiency of fermentation because soluble fraction of forages are readily digested and therefore accelerate the subsequent processes of digestion. The fact that absorption of nutrients mostly occur in the lower digestive tract, therefore changing the site of digestion may also affect the efficiency of feed utilization. This is true especially for the protein, fat or mineral fraction in which the digestion may be moved to the lower digestive tract by protecting the protein, fat of mineral entity so that they will not be digested in the rumen but still hydrolysable in the lower tract.

Modifying ruminal microbial metabolism of fatty acid in rumen through animal diet formulation is an effective way to enhance these functional fatty acids in ruminant-derived food products. However, it requires an understanding of the interrelationship between supply of lipid through the diet and rumen fermentation. Lipid in ruminant diets undergo extensive hydrolysis and biohydrogenation in the rumen. Apparent transfer efficiency of eicosapentaenoic acid and docosahexaenoic acid from feed to milk was very low (1.9 to 3.3%), which was, to a large extent, related to their extensive biohydrogenation in the rumen. Therefore, feeding a rumen-protected supplement containing eicosapentaenoic acid and docosahexaenoic acid, could be used to bypass the rumen (Or-Rashid et al. 2009).

Energy to protein ratio in rumen metabolism

The balance contents of the dietary energy and protein with further consideration of undegradability in the rumen may increase the efficiency of animal production. Synchronized availability of ammonia and energy in the rumen increased the microbial protein synthesis by which will increase the animal productivity in terms of meat or milk. The efficiency of microbial protein synthesis varied depending on the balance between ammonia and energy availability in rumen and it averages 30 g microbial N per kg fermented organic matter. There was a linear increase in final body weight and average daily gain (ADG) and a trend for a linear increase in dry matter intake (DMI) associated with increasing intake of degradable protein (DIP) concentration within the 5.1% intake of undegradable protein (UIP) treatments. Feed efficiency and net energy (NE) recovered from the diet were not influenced by dietary DIP concentration (Wagner et al. 2010). Certain rumen microorganisms were sensitive to a shortage of nitrogen; rumen concentrations of ammonia were 49% lower when the low-protein (LP) diets were consumed as were total bacteria (-13%), anaerobic fungi (-28%), methanogens (-27%), protozoa (-19%), cellulolytic bacteria and microbial diversity compared with when the high-protein (HP) diets were consumed. As a result, the digestibility of the LP diets was less than that of the HP diets. These findings demonstrated that the rumen microbial ecosystem is directly linked to the rumen fermentation pattern and, to some extent, to the efficiency of diet utilization by dairy cattle (Belanche et al. 2012).

Digestible versus less digestible protein

Positive effects of feeding more of the rumen undegradable protein (RUP) increased feed efficiency and fat corrected milk (FCM) plus 1.8 kg/d greater FCM and 0.08 kg/d greater fat, but milk protein content was lower and milk urea N and urinary urea excretion were elevated. Supplementation with rumen-protected methionine (RPM) increased DM intake 0.7 kg/d and FCM and fat yield by 1.4 and 0.06 kg/d and tended to increase milk fat content and yield of milk and protein (Broderick et al. 2009).

The utilization of condensed tannin as an additive in beef cattle diets with high level of concentrate and soybean meal as a source of true protein implies positive effects on crude protein utilization, decreasing digestion rate and ruminal digestibility of crude protein and consequently increasing the levels of metabolizable protein, with no changes in the ruminal fermentation parameters (Mezzomo et al. 2011). Meanwhile, balancing rumen degradable peptide supply to predicted requirement of ruminal microflora in steers improved fermentation efficiency and microbial output, which in turn improved animal performance (Brooks et al. 2012).

Products of livestock

Meat, milk or works are the primary products of livestock with concomitant yield of hide, fertilizer,

biogas, or young stocks (offspring). The efficiency of livestock production is affected by the nutritive quality of feed. Gain over feed cost may be more important to consider as an indicator of the economic advantage of keeping livestock. Feed is the major portion of livestock production costs; therefore, reducing feeding cost is prime interest to increase the benefit. The value of feed conversion ratio (FCR) may be used as an indicator to describe the efficiency of the feed utilization. A better quality of feed may produce higher quality livestock products in terms of nutritive value such as protein content of meat or total solid content of milk.

ADVANCED FEEDING MANAGEMENT

Farmers can play a role in each step of digestion and metabolism processes to improve the efficiency of feed nutrient utilization. The intervention of farmers to the digestion process is called as beyond usual feeding management because farmers can change the physiological process and manipulate the rumen ecosystem and along the digestive tract or even at the stage of metabolism in tissue level.

An example of the already well-known interventions of human to the rumen ecosystem is the one that reducing the protozoa population, which is known as defaunation; either partial or complete defaunation. The advantage of defaunation is indicated by the increased bacteria due to the reduced protozoa that acts as predator for bacteria. Increasing the number of bacteria will increase the fibrous feed digestion and therefore increase the availability of energy in the form of volatile fatty acids and energy yielding reaction through the proton-electron transport mechanisms. Saponins can be used as primary component of defaunator agent because saponins will disrupt the protozoa cell membrane and resulting in killing the protozoa. Defaunation may be carried out by selective removal of protozoa from the rumen microbial ecosystem by a cell membrane cholesterol-saponin interaction, which causes cell rupture, increases the nitrogen utilization of the ruminant, and may lead to an increase in growth, milk, or wool production. The growth-promoting effect was evident in the high roughage diet suggesting that the application of saponins or saponin-containing plant materials may be beneficial for the subsistence farmers (Wina et al. 2005). Microbial diversity in the rumen ecosystem enhances the resistance of the network of metabolic pathways present, as well as increasing the potential number of new pathway available (Edwards et al. 2008).

Development of feed technology

To support the feeding system beyond traditional feeding management, we needs the development of technology associated with the efforts to improve the nutritive value of feedstuffs, either before or after being fed to the animals (Table 1). Ensilage and fermentation of feedstuffs are mainly to improve the nutritive value of feedstuffs before being fed to the animals. Supplementation with ionophore antibiotics, secondary compounds of plants such as saponin, tannin and other bio-active substances, rumen modifier and essential nutrients is targeted to improve the nutrient utilization after the feeds are taken by the animals. Technology related to the modification of physiological processes of feed utilization should be developed and should be appropriate for the local specific condition.

Supplementation of polyether ionophores such as monensin, lasalocid, gramicidin, salinomysin and valinomysin may manipulate the efficiency of fermentation for a better utilization of nutrients. The ionophores act as modifier in the transport of ions across biological membrane. Ionophores are antimicrobial compounds that are commonly fed to ruminant animals to alter the microbial ecology of the intestinal microbial consortium, resulting in increased carbon and nitrogen retention by the animal and hence increasing production efficiency (Callaway et al. 2003).

The effects of monensin and lasalocid have been compared, and the additive response of malate in rations containing ionophores was evaluated using lambs (Gonzalez-Momita et al. 2009). The dietary treatments were: (1) Diet with 30 parts per million (ppm) lasalocid; (2) Diet with 30 ppm lasalocid and 0.3% malate; (3) Diet with 30 ppm monensin; and (4) Diet with 30 ppm monensin and 0.3% malate. Lambs fed diets with monensin had a 10.9% lower dry matter (DM) intake than those fed lasalocid. Malate had no effect on DM intake. Lambs fed monensin had lower NDF intake (37.8%) than those fed lasalocid. When malate was included in the diet, NDF intake was also reduced by about 25%. However, no difference in NDF digestibilities was observed between ionophores or by the addition of malate. Nitrogen balance was greater for lambs fed lasalocid diets, which had a higher crude protein intake, than lambs fed diets with monensin. Lambs fed diets with monensin or malate consumed less NDF and needed less time compared to those fed lasalocid. Lambs on the lasalocid diets consumed more fiber, which might be attributed to a greater selection of fibrous components of the diet (Gonzalez-Momita et al. 2009).

Feeding readily available nutrients in the rumen may also modulate the rumen function by affecting the

Table 1. Technology for advanced feeding management

Technology	Suggested mechanism	Qualitative response	References
Ensilage fermentation	Preserved nutrients; early degradation	Better nutritive value; higher *Lactobaccilus* concentration; lower pH	Gao et al. (2008)
Supplements of ionophores	Transport across membrane; modification of microbial activity	Increased nitrogen retention; increased animal growth rate	Callaway et al. (2003); Gonzalez-Momita et al. (2009)
Solubles nutrients	Early nutrient availability for rumen microbial use	Improved microbial activity	Benchaar et al. (2008)
Protection	Reducing rate of degradation; replacing site of digestion	Increased feed efficiency	Biricik et al. (2006)
Probiotics	Enzyme modification	Improved feed utilization	Wallace et al. (2008)
Encapsulation	Replacing site of digestion	Improved growth rate; essential fat composition	Or-Rashid et al. (2009); Wina et al. (2005)
Supplements of enzymes	Inducing early digestion	Better digestibility in the rumen	Wallace et al. (2008)
Supplement of sugar	Early energy supply for microbial activity	Improved microbial enzyme activity	Firkins (2011)
Chelation of minerals	Membrane transfer; immune response	Improved growth rate and production	Overton & Yasui (2014); Spears (1996)
De-oiled feedstuffs	Reduce fat effect on microbial activity	Improved feed nutritive value	Sereewatthanawut et al. (2008)

microbial synergism by which efficiency of nutrient utilization can be improved. The use of forage liquor rather than the entire forage material may also modify the energy utilization since forage liquor is to be digested much easier by microbial enzyme systems as compared to the whole forage particle. Benchaar et al. (2008) indicated that plant extracts containing of essential oils that have antimicrobial properties that make them potential alternatives to antibiotics to manipulate microbial activity in the rumen. Over the last few years, a number of studies have examined effects of essential oil and their active components, on rumen microbial fermentation (Benchaar et al. 2008).

Modifying the site of digestion for dietary protein may increase the availability of amino acids for animal tissue metabolism. Therefore a technique of protection of dietary protein from rumen degradation has been developed for the highly soluble protein feedstuffs, such as palm kernel cake and soy bean meal (Haryanto 2014). Similar technique can also be developed for essential fatty acids such as linoleic and linolenic acids. The unsaturated long chain fatty acids are important in affecting energy utilization in ruminants. Several seed oils are important sources of unsaturated fatty acids such as safflower oil and sunflower oil. Ruminal ammonia-N concentrations were not affected from the degradability characteristics of protein. Rumen pH and acetate: propionate were higher in diets containing slowly degradable starch than in diets rapidly degradable starch. Propionic acid was higher in diets containing rapidly degradable starch than in diets containing slowly degradable starch. The experiment failed to conclude that fermentation and utilization of nutrients in the rumen were affected by starch degradability more than protein degradability. Synchronizing starch and protein degradation in rumen had no effect on the intake and digestibility of nutrients in sheep (Biricik et al. 2006).

Supplementation of readily soluble nitrogen such as urea to the rumen ecosystem increases the concentration of ammonia (NH_3) which is required for the microbial protein synthesis. The use of urea in the diet, however, should be limited to a low level, i.e., less than 1% in the diet to prevent the occurrence of ammonia toxicity. Urea supplementation of feed for ruminants at doses up to 1% of complete feed DM is considered safe when be given to animals with a well adapted ruminal microbiota and fed diets rich in easily digestible carbohydrates. The substitution of protein by urea in well balanced feed for ruminants would not result in an increased environmental nitrogen load (EFSA 2012). The short chain fatty acids (SCFA) absorption also accelerates urea transport into the rumen, which via ammonium recycling, may remove protons from rumen to the blood. Ammonium absorption into the blood was also stimulated by luminal SCFA, indicating that the interacting transport processes for SCFA, urea, and ammonia represented evolutionary adaptations of ruminants to actively coordinate energy fermentation, protein assimilation,

and pH regulation in the rumen (Aschenbach et al. 2011).

Probiotics, which were defined as directly-fed microorganisms may be supplemented into the diets to manipulate the rumen microbial population, and therefore affect the enzyme production. The use of probiotics to improve the fibrous feed material digestion may be carried out prior to giving the feeds to the animal or by mixing it to the diets. Rumen microbes collected from slaughter houses can be used as preparation materials to produce probiotics under special processing management. Recent research on feed additives of natural or biological origin indicated that beyond replacement of growth-promoting antimicrobial feed additives in ruminant livestock production, it has potential commercial roles (Wallace et al. 2008).

Encapsulation of essential nutrients such as long chain unsaturated fatty acids, high protein concentrates, minerals and antioxidants may be beneficial to increase the efficiency of feed utilization, either biologically or economically. Recent experiments indicate that supplementation of protected conjugated linoleic acid reduced heat production and therefore increase the efficiency of energy utilization. Protected conjugated linoleic acid also reduces milk fat synthesis and increase the concentration of unsaturated fatty acids in the milk fat. Therefore, feeding a rumen-protected supplement containing eicosapentaenoic acid and docosahexaenoic acid, could be used to bypass the rumen (Or-Rashid et al. 2009).

Induction of cellulose degrading enzymes by cellobiose supplement in the rumen has been successful in increasing the utilization of energy as indicated by improvement of the cellulose digestibility by which more volatile fatty acids could be produced from the microbial fermentation activities. Cellobiose is a unit of 2 glucose monomers that can be obtained from the degradation of cellulose especially those from early emerging sprouts. Current scientific evidence suggests there is significant potential to use plants to enhance animal health in general and that of ruminants in particular. Active areas of research for plant bioactives (particularly saponin and tannin containing plants) include reproductive efficiency, milk and meat quality improvement, foam production/bloat control and methane production. Nematode control was also a significant area of research and the evidence suggests a much broader range of phytochemicals may be effective (Rochfort et al. 2008).

Supplementation of sugar solution increased the microbial enzyme activity which is required for the initiation of the degradation of cellulose. It suggested that the use of sugarcane shoots liquor which is high in sugar content may be a good strategy to increase the fibrous feed digestion. Sweet grasses contain high concentration of sugar as well, and this variety of grass has the potential to be developed in the future. In addition, the forage liquor could be used for bio-ethanol production while the residual from fermentative process was used as feedstuff for ruminants. Montagne et al. (2003) mentioned that diet had an important influence on gut health, including effects on proliferation of pathogenic bacteria and it could provide either beneficial or harmful input. Dietary fiber was a dietary component that had a major influence in this regard. There was evidence that some components of dietary fiber may improve gut health or alternatively enhance gut perturbation and subsequent diarrhea in young animals (Montagne et al. 2003).

Minerals are required by ruminants for microbial chemical reactions and for normal physiological processes of the animal. The biological value of minerals is affected by their conformation and ionic form. In many instances, the organic minerals are more effective than the inorganic ones such as zinc, copper and manganese. Chelation of those minerals with amino acids is already commercially marketed. A mixture of minerals which is specifically enriched with the essential elements may be given as mineral licks or mixed into the ration formula. The addition of one or more organic trace minerals to cattle diets has increased growth, milk production, reproduction and/or immune response in some studies. Zinc methionine has been studied to the greatest extent of any of the chelated or metal complex products available. Based on apparent absorption or tissue and blood concentrations, little evidence was available to suggest that organic trace minerals were considerably better absorbed than inorganic forms (Spears 1996). Trace minerals such as Zn, Cu, Mn and Se are essential with classically defined roles as components of key antioxidant enzymes and proteins. Available evidence indicates that these trace minerals could modulate aspects of oxidative metabolism and immune function in dairy cattle, particularly during the transition period and early lactation (Overton & Yasui 2014).

Ahola et al. (2004) have reported using crossbred, multiparous beef cows to evaluate the effects of Cu, Zn and Mn supplementation and source on performance in grazing cattle in eastern Colorado over a 2-year period. The results were not conclusive since in Year 1, the weight of calf weaned per cow were greater in controls than in supplemented cows and was greater in inorganic than organic treatments; however, opposite condition was found in year 2, indicating that supplementation and source of trace minerals affected the performance of beef cows in grazing system (Ahola et al. 2004).

De-oiled crop byproducts such as rice bran which contains fat approximately 15% of dry matter can be carried out leaving behind a higher protein content

residue that have a higher nutritive value for ruminants. De-oiled rice bran by hydrolysis in sub-critical water (SW) in the temperature range between 100 and 220°C for 0-30 minutes suggested that SW could effectively be used to hydrolyze de-oiled rice bran to produce useful protein and amino acids (Sereewatthanawut et al. 2008).

Beta hydroxybutyric acid (BHBA), one of ketone bodies resulted from long chain fatty acids oxidation, is important in the energy balancing such as for milk fat synthesis in ruminant. Poly-beta-hydroxybutyric acid (PHB) was produced from xylose and lactose using *Pseudomonas cepacia*. Approximately 50% PHB (grams of PHB/grams of biomass total) was produced (Young et al. 1994).

Feed blocks may reduce the use of concentrate feeds, thus reduce feeding cost and increase farmer's income. In addition to their nutritional and economical benefit, the success of feed block technology depends on its adoption by smallholders. Therefore, the active participation of farmers in the evaluation and transfer of feed block technology targeting their specific conditions was highly recommended (Ben Salem & Nefzaoui 2003).

Suggestion and recommendation

Based on the present feeding system of livestock, it is suggested that practical feeding management of ruminants should include the recent technology in order to increase the efficiency of feed utilization. Providing nutrients to support maximal production performance of livestock closed to the genetic potency may adopt the recently developed technologies as long as practicable and economically appropriate to the local condition.

It is recommended that further development of technology through research associated with modification of digestion, fermentation, absorption and metabolism of nutrients should be carried out to increase the efficiency of feed utilization which should be followed by improving the animal product quality and economic efficiency.

CONCLUSION

Conventional feeding system by allowing the animal to find their own nutrient requirements under the existing resource condition, such as in grazing land or under estate crop area, may be inadequate to satisfy the nutrient requirement due to the relatively low nutritive value of the available feedstuffs. Intervention of livestock raisers to increase the efficiency of feed utilization could be carried out by applying the currently available technologies to modify the physiological digestion, fermentation, absorption, and metabolism of nutrients. Ration formulation using pretreated feedstuffs before being fed to the animals opens the possibility to elevate the livestock productivity to the maximum potency. Application of appropriate technologies to the existing local farming condition should be developed in line with efforts to increase livestock production.

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INDEKS SUBJEK

SUBJECT INDEX

INDEKS PENULIS (*AUTHOR INDEX*) WARTAZOA VOLUME 25

UCAPAN TERIMA KASIH

Dewan Penyunting Wartazoa mengucapkan terima kasih dan penghargaan yang setinggi-tingginya kepada para pakar yang telah berperan sebagai mitra bestari pada penerbitan Wartazoa Volume 25 Tahun 2015, masing-masing kepada:

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Semoga kerjasama yang baik dapat terus berlangsung di masa-masa yang akan datang untuk lebih meningkatkan kualitas Wartazoa.

PEDOMAN BAGI PENULIS

KETENTUAN UMUM

Naskah yang dikirim belum pernah diterbitkan dan dalam waktu yang bersamaan tidak disampaikan kepada media publikasi lain. Perlu menandatangani surat pernyataan tentang keaslian naskah dan hak publikasi.

RUANG LINGKUP

Buletin ilmiah ini memuat tulisan hasil tinjauan, ulasan (*review*), kajian kebijakan dan gagasan serta pemikiran sistematis. Topik yang dibahas berupa informasi baru dan/atau memperkuat hasil temuan sebelumnya. Buletin ini diterbitkan 4 (empat) kali dalam setahun pada bulan Maret, Juni, September dan Desember.

PENGIRIMAN NASKAH

Naskah ditulis dalam bahasa Indonesia atau bahasa Inggris, ditulis dengan jarak 1,5 spasi, kecuali 1 spasi untuk Judul, Abstrak, Tabel, Gambar dan Lampiran. Jumlah halaman dalam naskah maksimum 20 halaman. Batas tepi kiri 4 cm dan masing-masing 3 cm untuk batas tepi kanan, atas dan bawah. Naskah diketik dengan jenis huruf *Times New Roman* dan ukuran (*font*) 12, menggunakan program *Microsoft Word*, kecuali program *Microsoft Excel* untuk tabel dan grafik serta *format* JPEG atau TIFF pada gambar (dalam format yang dapat diedit). Naskah lengkap dikirim melalui email dengan alamat wartazoa@yahoo.co.id. Bagi naskah yang diterima, penulis berhak menerima 1 (satu) buletin asli dan 10 (sepuluh) eksemplar cetak lepas.

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1. **Judul** ditulis singkat, jelas, spesifik dan informatif yang mencerminkan isi naskah serta tidak lebih dari 15 kata.
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3. **Abstrak** ditulis dalam bahasa Indonesia dan bahasa Inggris dan merupakan intisari naskah, masing-masing tidak lebih dari 250 dan 200 kata yang dituangkan dalam satu paragraf dengan jarak satu spasi.
4. **Kata kunci** (*key words*) dalam bahasa Indonesia dan Inggris, boleh kata tunggal dan majemuk, serta terdiri atas tiga sampai dengan lima kata.
5. **Pendahuluan** terdiri dari latar belakang, permasalahan atau rumusan masalah, serta tujuan dan manfaat ulasan (*review*).
6. **Isi pokok bahasan** menyajikan dan membahas secara jelas pokok bahasan dengan mengacu kepada tujuan penulisan.
7. **Kesimpulan** merupakan substansi pokok bahasan yang menjawab permasalahan serta tujuan penulisan dan bukan merupakan tulisan ulang atau ringkasan dari pembahasan.
8. **Saran** (apabila ada) dapat berisi rekomendasi, tindak lanjut atau implikasi kebijakan atas kesimpulan yang diperoleh.
9. **Ucapan terima kasih** (kalau ada).
10. **Daftar pustaka**:
    a. Minimal 25 acuan, diutamakan menggunakan pustaka 10 tahun terakhir dan minimal 80% pustaka primer. Sitasi hasil penulisan sendiri paling banyak 30% dari total acuan.
    b. Pustaka dari internet hanya diperbolehkan dari sumber yang dapat dipertanggungjawabkan seperti jurnal, instansi pemerintah atau swasta.
    c. Nama pengarang disusun secara alfabetis dan tahun penerbitan.
11. **Tabel:**
    a. Huruf standar yang digunakan adalah *Times New Roman* dengan jarak satu spasi dan *font* 11.
    b. Judul adalah kalimat singkat, jelas, dapat dimengerti tanpa harus membaca naskah.
    c. Setiap kolom dari tabel harus memiliki tajuk (*heading*). Unit harus dipisahkan dari judul dengan koma dalam kurung atau di bawahnya.
    d. Keterangan tabel ditulis di bawah tabel dengan jarak 1 spasi dan *font* 11. Sumber data dituliskan di bawah tabel atau di dalam tabel pada tajuk sendiri.
    e. Garis pemisah dibuat dalam bentuk horisontal.

    Contoh tabel:

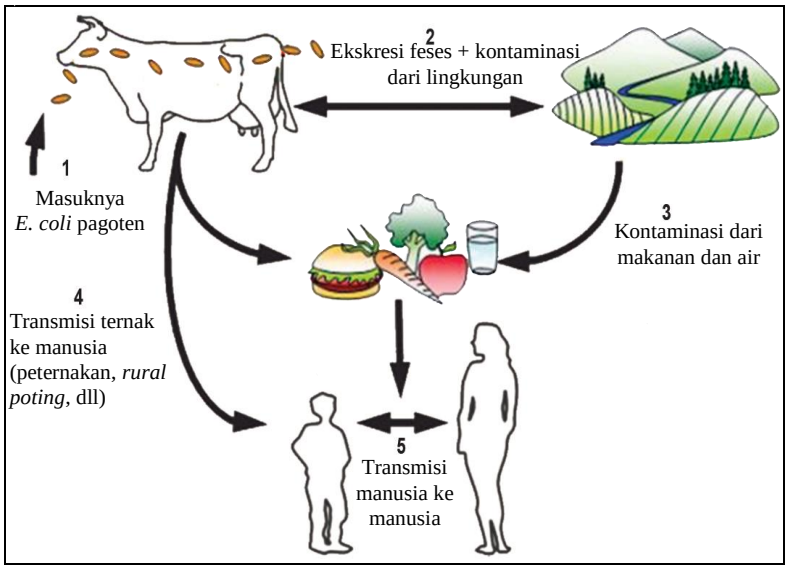
Tabel 1. Respons kambing terhadap berbagai bentuk fisik pakan komplit

Bentuk pakan komplit	Genotipe kambing	Umur (bulan)	Konsumsi (% BB)	PBBH (g)	NKR[a)]	Kecernaan (%)	Sumber
Pelet	Jamunapari	24-27	3,0-4,0	154-192	5,2-8,4	65	Srivastava & Sharma (1998)
Cacah[b)]	Alpine	9-36	4,4	102	11,0	tt	Galina et al. (1995)
Cacah	Nubian	9-36	4,1	85	11,0	tt	Galina et al. (1995)
Tepung kasar	Boerka	3-6	3,9-4,9	71-89	11,2	62-81	Ginting et al. (2007)
Tepung kasar	Afrika	16-18	5,8	50-58	10-13	68-78	Areghero (2000)

[a)]NKR: Nilai konversi ransum (konsumsi/PBBH; g/g); [b)]Pakan dasar dalam bentuk cacahan dan konsentrat dalam bentuk tepung; tt: Data tidak tersedia

12. **Gambar dan grafik:**
    a. Judul menggunakan *Times New Roman* dengan jarak 1 spasi dan *font* 11, berupa kalimat singkat dan jelas diletakkan di bawah gambar dan grafik.
    b. Garis pada grafik harus secara jelas terlihat perbedaan satu dengan yang lain apabila terdapat lebih dari satu kurva.
    c. Gambar dengan kontras yang jelas dengan ukuran yang proporsional dan beresolusi tinggi agar dapat tampil baik untuk penampilan terbaik.
    d. Tuliskan sumber gambar/grafik di bawah judul.

    Contoh gambar dan grafik:



Gambar 3. *Food borne disease E. coli* O157:H7

Sumber: Scieh (2001) yang dimodifikasi

13. **Satuan pengukuran:** dipergunakan sistem internasional (SI)**.**
14. **Penulisan angka desimal:** untuk bahasa Indonesia dipisahkan dengan koma (,), untuk bahasa Inggris dengan titik (.).
15. **Penulisan pustaka dalam teks:**
    a) Penulisan pustaka mengacu pada Council of Science Editor (CSE) edisi ke-7 tahun 2006.
    b) Pustaka harus ditulis nama penulis terlebih dahulu, diikuti tahun, contoh: Diwyanto (2007) atau (Diwyanto 2007).
    c) Bila ada dua nama penulis dalam satu makalah, maka nama penulis harus ditulis semua, contoh: Albenzio & Santilo 2011.
    d) Bila ada lebih dari dua nama penulis dalam satu makalah, maka harus ditambah et al. (huruf tegak dan diberi titik di belakang huruf), contoh: Jayanegara et al. (2012) atau (Jayanegara et al. 2012), dan di dalam daftar pustaka ditulis hingga penulis kesepuluh serta diakhiri dengan et al..
    e) Bila ada lebih dari satu pustaka untuk satu pernyataan, maka harus ditulis urutan dari tahun yang tertua dan urutan alfabet nama penulis bila tahunnya sama, contohnya: (Diwyanto et al. 2007; Jayanegara et al. 2012; Wina 2012).
    f) Pustaka dalam pustaka seperti contoh: Teleni dalam Widiawati (2012) tidak diperkenankan.
    g) Bila suatu pernyataan diperoleh dari komunikasi pribadi, perlu dicantumkan "nama orang yang dihubungi" dan diikuti dengan (komunikasi pribadi) di belakangnya.
    h) Memuat nama penulis yang dirujuk dalam naskah.
    i) Jika penulis yang sama menulis lebih dari satu artikel dalam tahun yang sama dapat dibubuhi huruf kecil.
    j) Disusun secara alfabetis dan tahun penerbitan menurut nama penulis.
16. **Cara penulisan pustaka di dalam Daftar Pustaka**:
    a) Setiap pustaka yang disebut di dalam tulisan harus dimasukkan ke dalam Daftar Pustaka yang ditulis di bagian akhir makalah.
    b) Pustaka yang dirujuk harus dipublikasi dalam kurun waktu 10 tahun terakhir dengan proporsi pustaka jurnal minimum 80%.
    c) Pengutipan pustaka dari internet hanya diperbolehkan dari sumber yang dapat dipertanggungjawabkan seperti jurnal, instansi pemerintah atau swasta. Wikipedia tidak dapat dijadikan sumber pustaka.
    d) Pustaka dengan "Anonimus" tidak diperbolehkan.
    e) Bila tidak disebut nama penulisnya, maka yang di dicantumkan adalah nama institusi atau penerbit.
    f) Makalah yang sudah diterima tetapi masih dalam proses pencetakan, harus ditulis (*in press*) pada akhir pustaka.
    g) Beberapa contoh penulisan sumber acuan adalah sebagai berikut:

Buku:
Stiglitz JE. 2010. Free fall. New York (US): WW Norton and Company Inc.

Jurnal:
Kostaman T, Yusuf TL, Fahrudin M, Setiadi MA, Setioko AR. 2014. Pembentukan *germline chimera* ayam Gaok menggunakan *primordial germ cells* sirkulasi segar dan beku. JITV. 19:17-25.

Artikel dalam Buku:
Prawiradiputra BR. 2012. Tanaman penutup tanah untuk perkebunan kelapa sawit. Dalam: Tiesnamurti B, Inounu I, penyunting. Inovasi pengembangan sapi sistem integrasi sapi sawit. Jakarta (Indonesia): IAARD Press. hlm. 159-187.

Hanotte O, Han J. 2006. Genetic characterization of livestock population and its use in conservation decision making. In: Sannino J, Sannino A, editors. The role of biotechnology in exploring and protecting agriculture genetic resources. Rome (Italy): Food and Agriculture Organization of the United Nations. p. 89-96.

Internet:
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