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Poster presentation

P1 Surveillance of *Mycoplasma gallisepticum* Infection in Mixed Native Thai Chickens

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Introduction and Objective

Mycoplasma gallisepticum (MG) infection is known as a chronic respiratory disease (CRD) of poultry (7). Infected birds show respiratory signs including conjunctivitis and sneezing. MG infection can be diagnosed by 2 main methods: antigen detection and serology. Antigen detection can be done by culture technique and DNA detection by polymerase chain reaction (PCR) technique. Serology procedures can be tested by serum plate agglutination (SPA), hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA).

The objective of this study was to investigate the surveillance of MG infection in mixed native Thai chickens by serology and PCR technique.

Materials and Methods

Samples were submitted from 30 mixed native Thai chicken flocks in Nakornpathom province between September 2005 and October 2006. 15 birds per flock, aged between 1 and 4 months without MG vaccination were investigated. Individual birds with identification were bled at the wing vein and swabbed at the choanal cleft. Blood samples were separated for SPA (Nobilis®) and ELISA (ProFLOK®). Swab samples were inoculated into a Frey's broth medium supplemented with 15% swine serum (FMS) (5) for PCR technique described by Lauerman (6).

Results and Discussion

The numbers of positive flocks tested by SPA, ELISA and PCR were 7, 8 and 6 flocks, respectively. The percentage of positive results depending on age: 1 month, 1- 2 month, 2 - 3 month and 3 - 4 month tested by SPA, ELISA and PCR was 18.2% - 40%, 16.7% - 40% and 18.2% - 40%, respectively (Table1).

Table 1: Percentage of positive results depending on age: 1, 1- 2, 2 - 3 and 3 - 4 month tested by SPA, ELISA and PCR

Age (m.)	Numbers of flocks	Numbers of positive flocks (%)		
		SPA	ELISA	PCR
1	6	0	1 (16.7%)	0
1 - 2	11	2 (18.2%)	3 (27.3%)	2 (18.2%)
2 - 3	8	3 (37.5%)	2 (25%)	2 (25%)
3 - 4	5	2 (40%)	2 (40%)	2 (40%)

This study found antibody reactors and MG DNA at older than 1 month. The incubation period of those experimentally infected with MG organisms is 6 - 21 days (1, 2). Therefore, 1 month old birds did not obviously exhibit the antibody, evidence of MG infection or any clinical signs. This study also suggested that flocks had a high chance of infecting MG organisms after rearing the birds for 1 month or more. In addition to the high numbers of positive reactors in the older flocks, the respiratory signs were observed in some flocks. However, these flocks showed more severe respiratory signs if these birds were complicated with other organisms or live viral vaccines (4, 3).

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P2 Immunohistochemical Study of Natural Highly Pathogenic Avian Influenza Subtype H5N1 Infection in Avian Species

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Introduction and Objective

During November 2003, the outbreak of H5N1 highly pathogenic avian influenza (HPAI) involving commercial farm chickens occurred in Thailand and other south-east asian countries. The H5N1 HPAI was a rapidly fatal systemic disease in avian species with directly transmission from avians to man. In addition to man and avians, other mammals are also infected. Other free ranging birds were might be the host for the spread of the disease. Much experimental research on the H5N1 HPAI has presented the varied pathobiologic features on avian species (1,2). The objective of this investigation was to observe the pathology and tissue tropism in free ranging and captive avian species using the immuno-histochemistry (IHC) method.

Materials and Methods

Twenty five free ranging and captive birds (7 crows, 8 pheasants, a white peafowl, an ostrich, 3 layer hens, 3 quails, a creeper, and a goose) were necropsied and selected organs for histopathology were collected and fixed in 10 % neutral buffered formalin, routinely processed and embedded in paraffin. Sections were made at 4 μm and stained with hematoxylin and eosin (HE). For IHC study, the sections were treated with 1% Proteinase K followed by 1 % bovine serum albumin. The sections were then incubated with a 1/300 dilution of a monoclonal antibody against the nucleoprotein of influenza A virus (HB65, EVL) followed by incubation with a biotinylated goat anti-rabbit IgG antibody. The presence of PCV-2 antigen was visualized by avidin-biotin complex peroxidase method (ABC kit) followed by an addition of DAB substrate.

Results and Discussion

Macroscopically, moderate to severe acute pulmonary congestion, edema and hemorrhage occurred in all birds. Most of the crows showed multifocal hemorrhage of the brain. Severe generalized hemorrhages of the visceral organs were found in the ostrich. Prominent histopathology in all the birds was observed in the lungs, brain and pancreas, some of which were severe diffuse acute exudative interstitial pneumonia, severe multifocal to diffuse necrotizing encephalitis and pancreatitis, respectively. The presence of the antigen in these tissues was strongly positive, unless other tissues were varied among the birds. The distribution of the antigen was more widespread than the histopathology. In the brain, neurons, glia and ependymal cells were positive in nuclei and cytoplasm. In other tissues, the antigen was identified in the nuclei and cytoplasm of many cell types of organ examined. The specific parenchymal cells of each organ consistently containing viral antigens was reported (1). Of particular interest was the detection of antigens in the kidney; the intranuclear inclusion-like lesion of renal tubular cells were strongly immuno-positive. Among the species, the severest lesions and more widespread immunostain were in the crows, the peafowl and the ostrich, while the lowest lesions were in the goose. The difference in the intensity and distribution of the antigen among the tissues and species probably depended on the severity and pathogenesis of the rapidly fatal disease. Therefore, in natural infections of birds, virus load, ability of viral replication and the host resistance, may be involved in the distribution of antigens. Since the crow is a scavenger bird it might be infected with a high concentration of virus from the infected dead bodies.

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P3 Estimation of Additive and Maternal Genetic Effects on Egg Production in Native Thai Chickens (Praduhangdum)

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Introduction and Objective

Although the maternal genetic effect is not important for egg production traits (2), excluding the maternal genetic effect leads to an overestimation of direct heritability estimates (5). The aims of this study were to determine the most appropriate model for the data set used and to estimate additive genetic and maternal genetic effects on egg numbers of native Thai chickens (Praduhangdum)

Materials and Methods

Data consisted of records of total egg numbers over a one year period (EN) from 287 native Thai chickens. The animal models used to estimate the variance component were:

Model [A0]: $y = X\beta + Za + Zm + e$
with Cov(a, m) $= 0$
Model [A]:y $= X\beta + Za + Zm + e$
with Cov(a, m) $= 0.15$
Model [B]: $y = X\beta + Za + e$

where y was the vector of observation; β, a , m and e were the vectors of fixed effects (hatch, month of birth, age at 1st egg), additive genetic effect, maternal genetic effect and residual effect, respectively; X, Z were incidence matrices relating observation to β, a and m, respectively. The REMLF90 package[4] was used to estimate variance components and heritabilities. Log likelihoods (-2log L) were compared by using a chi-square test (1).

Results and Discussions

The average ± standard deviation (S.D.) of egg numbers was found to be 131 ± 49.23 eggs. Results in Table 1 indicated that Model [B] might be the most fitting model because of its log L values and residual variance (δe^2) were the lowest. The direct heritability estimate was 0.37±0.03 (h_a^2±S.E.). This estimate was higher than the average value of 0.10 in native Thai crossbred reported by Suparat (2002). The maternal heritability estimates (h_m^2±S.E.) were 0.04 ± 0.01 and 0.02 ± 0.004 in model [A0] and [A], respectively. Table 2 showed that fitting model [B] improved the -2 log L significantly (p<0.01) when compared to model [A] and model [A0].

Table 1 Estimates of (co)variance components, heritabilites ± standard error (S.E.), -2log L values for egg numbers.

	Model		
	[A0]	**[A]**	**[B]**
δa^2	665.00	675.00	820.00
δm^2	84.60	36.10	-
δa, m	0.00	42.60	-
δe^2	1470.00	1460.00	1400.00
h_a^2±S.E.	0.30±0.02	0.30±0.02	0.37±0.03
h_m^2±S.E.	0.04±0.01	0.02±0.00	-
-2log L	2341.54	2341.54	2318.04

Table 2 Likelihood ratio test (LRT) and chi-square test for comparing these models.

Models	LRT
[A]-[B]	- 47.0106**
[A]-[A0]	- 0.0032
[A0]-[B]	- 47.0138**

** P<0.01

The obtained results indicated that maternal genetic effects should be ignored. The reason that the maternal genetic effect was not significant could have been because of the small w-chromosome (3). It should be concluded that maternal genetic effect is not important for egg numbers.

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P4 Investigations of Ampullary Oviductal Epithelium in Repeat Breeder Gilts by Scanning Electron Microscope

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Introduction and Objective

Gilt fertility impacts herd performance because they are the largest farrowing group and 35-55% of the sow herd is replaced by gilts each year. Reproductive problems are the main reason for culling gilts in swine herds and failure to conceive is the most frequent problem[1]. The oviduct plays an important role in sperm transport, oocyte maturation, fertilization and early embryo in development (2). There are several factors that disturb these events in the oviduct causing repeat breeding. Previous studies have reported the pathological changes and the decrease of epithelial cell heights in repeat breeder gilt ampulla. The aim of the present study was to examine the epithelium of repeat breeder gilt ampulla compared to cyclic sows by scanning electron microscope (SEM).

Material and Methods

The oviducts of 9 replacement gilts (Landrace x Yorkshire) culled due to repeat breeding were collected from commercial swine farms. The ampullary segments were dissected and fixed in glutaraldehyde. The specimens were examined by SEM and sow oviducts at follicular (n = 3) and luteal phases (n = 3) were used as a normal group.

Results and Discussion

The ciliated cells of the sow ampulla at the follicular phase were evenly distributed on the epithelium and their cilia extended above the secretory cells (Fig. 1a), whereas the ampulla at the luteal phase were entirely covered with the bulbous processes of secretory cells (Fig. 1b) which is similar to Abe and Oikawa (1992) (3). The repeat breeder gilt ampulla at the follicular phase also showed numerous ciliated cells but their cilia revealed an atypical structure and had inflammatory cell infiltration (Fig. 2a, b). At the luteal phase, the bulbous processes of gilt ampulla were disrupted, because irregular in shape and debris was found on the epithelium (Fig. 2c, d).

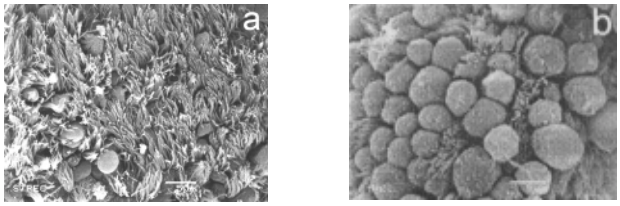

Figure 1 SEM photomicrographs from the luminal surface of normal sow ampulla at the follicular (a) and luteal phases (b).

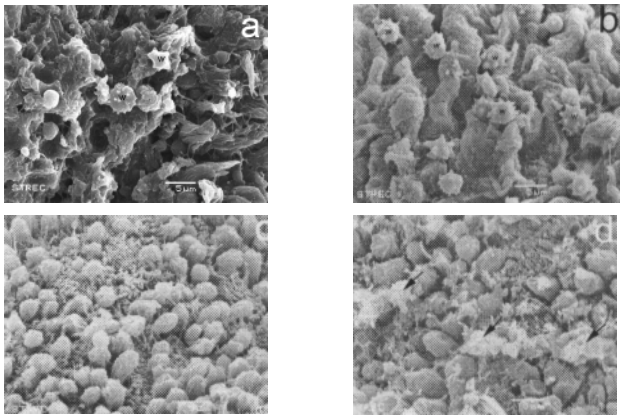

Figure 2 SEM photomicrographs from the luminal surface of repeat breeder gilt ampulla at the follicular phase (a, b) and luteal phase (c, d). The arrow heads point to some mucous debris on the epithelial cells. w, white blood cell.

The results indicated that the ultrastructural changes of the epithelial surface were found in repeat breeder gilt ampulla and differed from that in normal pigs. These abnormal epithelial changes might affect the proper function of repeat breeder gilt oviducts.

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P5 Development of a Multiplex Polymerase Chain Reaction Assay for the Development Diagnosis of Porcine Enteric Diseases

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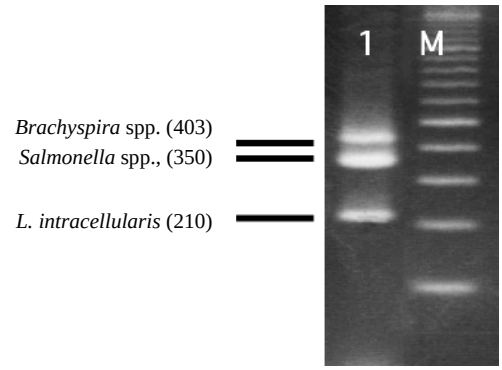
Introduction and Objectives

Lawsonia intracellularis, Salmonella spp., and *Brachyspira hyodysenteriae* are enteric pathogens causing porcine proliferative enteropathy, swine dysentery, and porcine salmonellosis. Rapid and sensitive methods are, therefore, required to detect and identify these bacteria. In the present study, we report the development, optimization, and performance of a multiplex PCR assay for simultaneously amplifying target DNA of these three enteric pathogens.



Materials and Methods

All three DNA templates obtained from the positive control bacteria strains at a concentration of $3.0x10^9$ CFU/ml of *Brachyspira hyodysenteriae*, $4.2x10^{15}$ CFU/ml of *Salmonella* spp., and $1.0x10^{5.5}$ $TCID_{50}$/ml of Lawsonia intracellularis, respectively. The *B. hyodysenteriae*-specific and *L. intracellularis*-specific primers were designed by Suh and Song (2005) to amplify a 403 and 210 base pair PCR fragments. A 350- bp fragment of the FimA gene of *Salmonella* spp. was synthesized.

The specificity of the primers for the three species was assessed by applying them to 15 enteric bacterial species. For a single PCR assay and the multiplex reaction of all three bacterial isolates were examined in which concentration of primers, Taq-polymerase, nucleotide $MgCl_2$, and the annealing temperature were optimized. The sensitivity test was determined with a different 10-fold diluted concentration of each bacterium, the DNA extracts, and then subjected to the PCR assay.

Results and Discussion

The multiplex PCR developed in this research was able to detect *L. intracellularis, Salmonella* spp., and *B. hyodysenteriae* simultaneously in a single reaction. The sizes of the amplified products as determined by agarose gel electrophoresis were 210 bp for *L. intracellularis,* 350 bp for *Salmonella* spp., and 403 bp for *B. hyodysenteriae*. No amplicons were produced from the water or *E. coli* negative controls. With a series of 10-fold dilutions, the lowest concentrations with positive single PCR results were $1.0x10^{0.5}$ $TCID_{50}$/ml for *L. intracellularis*, 4.2 x 10^3 CFU/ml for *Salmonella* spp., and $3.0x10^4$ CFU/ml for *B. hyodysenteriae*. The 210- and 350-bp PCR products, which are produced by two of three primers in a multiplex PCR reaction, is produced only from *L. intracellularis* and *Salmonella* spp., while the band did not appear from non target organisms. On the other hand, a 403-bp product produced by *B. hyodysenteriae* is not specific for the above target species.

In conclusion, the multiplex PCR has been proven sensitive, specific and cost effective and it can be useful in the diagnosis, screening and surveillance of diseased pigs. However, the multiplex PCR is not able to differentiate *Brachyspira* spp. from others. A further evaluation in fecal samples should provide a more convenient demonstration of the usefulness of this assay in the routine diagnostic laboratory.

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P6 Bacterial Contamination of Fresh and Extended Boar Semen

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Introduction and Objective

The success of artificial insemination is dependent on several factors i.e. semen doses, and insemination time. Several studies had shown the impact of semen quality on the reproductive performance of sows (Althouse et al., 2000). Semen collection in the boar is commonly contaminated with pathogens i.e. bacteria. The unfavorable effects of bacterial contamination in semen seem to be concentration dependent, both in semen quality and semen longevity. Antimicrobial drugs have been used as an essential element in semen extender compositions, to control bacterial growth. Time and environment are also critical components in the negative impact of bacteria on spermatozoa. The aim of this study was to investigate the bacterial contamination on fresh and extended boars semen in pig herds in Thailand.

Materials and Methods

Field studies on the bacterial contamination of fresh and extended semen were performed between July and October 2006, on 24 commercial pig herds in the central part of Thailand. All the pigs in the study were tended according to the guidelines of the Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand. The herd size ranged from 400-2,400 sows, and with 20-60 boars. Semen doses were produced from their own herd. After semen collection, sperm motility and sperm concentration were evaluated before mixing semen doses. Semen was diluted in a preservation medium and transferred into insemination bottles. All the herds were visited by the authors, for the collection of fresh and extended semen samples, and semen samples delivered to the laboratory. Semen was cultured on blood agar, and MacConkey agar for bacterial growth. Inspection and isolation were performed after approximately 24 and 48 h of incubation. Antibiotic sensitivity tests were performed using the Kirby-Bauer disk diffusion susceptibility method.

Results and Discussion

102 fresh and extended semen samples were collected from 24 pig herds. Bacterial contaminants and frequency of isolations are presented in Table 1. The most frequently isolated contaminant bacteria from the fresh and extended semen (89% and 31% of semen samples, respectively) were *Micrococcus* spp., *E. coli.*, *Proteus mirabilis*, and *Citrobacter* spp.. Most bacterial isolations from semen were found to be sensitive to gentamicin sulphate, penicillin-streptomycin and amikacin sulphate.

Table 1 Bacterial contamination isolated from semen samples (n = 102)

Bacterial isolate	Frequency of isolation (no.)	
	Fresh	Extended
Micrococcus spp.	45	12
E.coli	26	7
Proteus mirabilis	14	5
Citrobacter spp.	14	4
Pseudomonas aeruginosa	13	-
Staphylococcus epidermidis	12	3
Providencia spp.	10	2
No bacteria growth	11	70

The results of the present study show that high proportion of semen samples were contaminated both during semen collection and processing. Thus, the standard sanitation and hygiene methodologies specific to semen collection must be revised.

Acknowledgment

This study was supported by the Veterinary Science Research Fund (RG 26/2549), Chulalongkorn University

P7 A Field Trial of Dietary Fiber Supplementation: Effects on Fecal Characteristics, Reproductive Performance and Nutrient Digestibility in Crossbred Pigs

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Introduction and Objective

Fiber additions to sow diets have contributed to increased reproductive performance in sows and increased weaned pigs per sow by the way of; limiting body weight gain, preventing constipation, reducing health problems and stimulating the feed intake. The objective of the current study was to evaluate the effect of dietary fiber supplementation on the physico-chemical characteristics of feces, reproductive performance, and apparent nutrient digestibility in gilts and sows.

Materials and Method

A total of 96 non-pregnant crossbred pigs with half gilts and half sows were selected for this field trial. The trial composed of two periods; gestation and lactation. Treatments were isoenergetic diets that were composed of 2 levels of dietary fiber: control (5%) and supplement (5+2%). Pigs were fed *ad libitum* twice daily. Feces were collected for the measurement of nutrient digestibility using Cr_2O_3 as a marker. Fecal scores were graded twice daily during the study using the following scale: 1 = constipation, 2 = firm, 3 = moderately firm, 4 = soft, and 5 = loose. Proximate and Cr analyses were performed on feed and fecal samples. Measurements of reproductive performance were recorded including the number of pigs born alive, the litter birth weight, the number of pigs weaned, the litter weaning weight, and the litter body weight gain of the piglet. Data was analyzed as a complete randomized blocked design with 2x2 factorials.

Results and Discussion

Most pigs in this study either receiving the control or fiber-supplement diet had a fecal score of 2 indicating a firm stool which was normal. No difference was seen in piglet weaning litter size, piglets weaning body weight and litter body weight gain. Pigs fed the fiber-supplement diet appeared to have lower ($p< 0.01$) dry matter (DM), organic matter (OM), crude protein (CP), and crude fat (CF), but greater ($p< 0.01$) crude fiber digestibility when compared to pigs fed the control diet. Fiber has been shown repeatedly to decrease nutrient digestibility and as a consequence, increase fecal bulk and nutrient excretion (1,4). Quantification of fiber in swine diets is difficult due to the complexity and diversity of polysaccharides involved (2,3). It is suggested that the implications of such events could be considerable in the recommended-diet supplemented with an additional 2% fiber during gestation period in both gilts and sows to prevent their constipation before farrowing without affecting reproductive performances.

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P8 Financial Feasibility Study of Radio Frequency Identification (RFID) Implementation in Pig and Dairy Farms

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Introduction and Objective

Radio frequency identification (RFID) is a new technology that relies on the transfer of information through the radio waves. Nowadays, RFID implementation for livestock is a growing trend. It is playing an important role in the future of food security control. However, using the RFID system needs to be thoroughly investigated, because it might be the cause a negative impact on the profitability of the farm. Therefore, the purpose of this study was to evaluate the cost and benefit of using the RFID system at a farm level on pig and dairy farms. The results would facilitate farmers to make cost effective decisions in using this technology.

Materials and Methods

One pig and one dairy farm model were constructed with two farm plans, with and without RFID implementing project plans. The financial study was analyzed using a net incremental return from without project to with project. Financial feasibility indexes i.e., annual RFID system cost, net present value (NPV), benefit-cost ratio (BCR) and internal rate of return (IRR) were calculated as following formula. NPV was calculated by Σ (INBt / (1+r)t) where INBt was an incremental net benefit at t period, t was a period (t = 1,....,15 yrs) and r is a discount rate (8%). BCR was calculated by [Σ (IB_t/ $(1+r)^t$)]/[($ICt/(1+r)^t$)] where IB_t was an incremental benefit at *t* period, IC_t was an incremental cost at *t* period, *t* was a period (t = 1,....,15 yrs) and r is a discount rate (8%). IRR was calculated by [rL+(rH-rL)]x[NPV_L/ (NPV_L + NPV_H)] where NPV_L and NPVH were net present value by using low and high discount rate (rL and rH), respectively.

The additional inputs for the RFID system were variables, depending on the needs and specific goals for RFID implementation. The specific equipment for pig farms was electronic ear tags (passive type) and automatic feeders (1 set per 50-pig heads), and for dairy farms bolus electronic passive tag and milk flow meters (4 sets per 100-dairy cow heads). The common equipment for both pig and dairy farms were handheld readers, wireless network systems, computers and herd management software.

Results and Discussion

The RFID annual cost for a 100-head of pig and dairy cow farm was 306.32 and 254.27 baht per head, respectively. As the herd size increased, the annual cost per head decreased for the RFID fixed cost and total cost. For a 100-head pig farm, the results clearly implied that a real discount rate of 8 percent, NPV of net incremental return being as 33,235 baht, with BCR of 1.15, and with IRR of 9.1 percent. For the 100-head dairy farm, with a real discount rate of 8 percent, NPV of net incremental return was 26,517 baht, with BCR of 1.69, and with IRR of 12.5 percent. Moreover, at an increasing or a decreasing of incremental benefit of 5, 10, and 15 percent, respectively, the NPV, BCR, and IRR of farms with the RFID system were also better than farm without RFID system. These results imply that the implementation of a RFID system in both pig and dairy farms is a financially feasible project. The using of an RFID system might be an alternative way to improve farm productivity.

Acknowledgement

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P9 Correlation Between Plasma L-Carnitine Concentration and Age in Neonatal Piglets

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Introduction and Objective

Carnitine is an amino acid derivative which is synthesized from lysine and methionine as precursors *in vivo*. It is necessary for the transfer of fatty acids across the inner mitochondrial membrane for beta-oxidation metabolism. High levels of L-carnitine during the nursery phase improve growth performance. Generally, a sow's colostrum and milk are good sources of carnitine for suckling pigs. Therefore, the objective of this research was to determine the weekly change in plasma L-carnitine concentration over a 28 day suckling period.

Material and Methods

Five lactating sows were used in this study. Measurements of piglet body weight were recorded on the first day of age and subsequently on the 3rd day and every week after birth. Blood samples from piglets were taken weekly from the anterior venacava and put into K-EDTA tubes. The plasma samples for the L-carnitine analysis were assayed with a L-carnitine enzymatic UV kit by spectrophotometry at 340 nm wavelength. Significantly statistical analyses to determine the differences between age and L-carnitine levels as well as body weight changes were performed using ANOVA with a post hoc Student-Newman Keul multiple range test as a p value < 0.05. The linear relationship between suckling ages and L-carnitine concentration was determined by the spearman method.

Results and Discussion

The results showed that the L-carnitine concentration of piglets after birth was 3.94 ± 0.13 mg/L. Then, L-carnitine levels on the 7th day of age were significantly decreased ($p < 0.05$) compared to those on the 1st day of age. On the 14th day, L-carnitine concentration increased significantly ($p < 0.05$) again and maintained its level until the weaning day (Fig.1). Similar to the increase in the plasma L-carnitine profile after 1 week of suckling, the body weight changes also increased significantly ($p < 0.05$) (Fig.2). The linear regression of suckling ages and L-carnitine levels was significant ($p < 0.05$) and positive correlation (Fig.3).

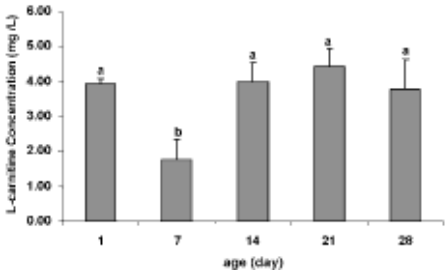

Fig. 1 L-carnitine concentration of piglets during the suckling period (means ± SD.).

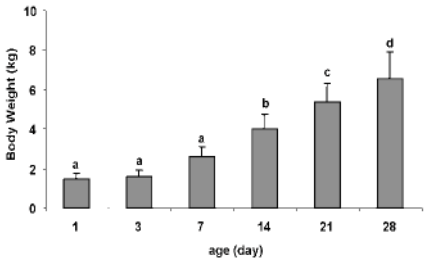

Fig. 2 Body weight changes of piglets during the suckling period (means ± SD.).

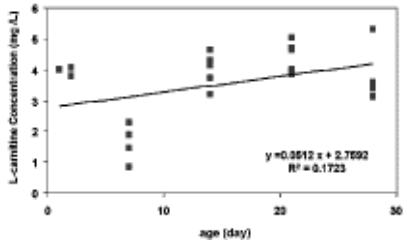

Fig. 3 Linear relationship between suckling ages and L-carnitine concentration.

Further investigations remain to be conducted into the molecular regulatory mechanism to transport L-carnitine from dams to their piglets via mammary tissue.

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P10 Relation Between Microorganisms, Lactic Acid Production, and Dye Reduction Tests in Raw Milk

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Introduction and Objective

Mastitis, milk handling, storage equipment and temperature influence the increase in the numbers of microbial contaminations of raw milk. Methylene blue reduction (MB) and resazurin (RR) tests which are used to determine of milk quality, based on the color change from the oxidation-reduction time and is theoretically related to aerobic mesophilic bacterial counts is still doubtful. However, the lactic acid production of certain bacteria is proposed as a measure for those counts. The study of the relation of microorganisms, lactic acid production and dye reduction tests are our objectives in order to develop a new tool closely related to the total bacterial count.

Materials and Methods

Sampling

A total of 360 raw bulk tank milk samples from Nakhornpathom and Prachuapkhirikhun provinces were investigated.

Dye reduction tests

Methylene blue reduction and resazurin tests were performed as previously described (3).

Titratable acidity and pH

The procedure described by Richardson (3) was used to access the acidity and pH of milk. The acidity was expressed as percentage of lactic acid equivalent.

Microbiological analysis

The milk was diluted and plated in duplicate. Total bacterial counts were carried out in a Plate Count agar, Staphylococci in a Baird Parker agar, Streptococci in an Edward's medium (Oxoid) and coliform counts in a Violet Red Bile agar (Difco)(1,2).

Statistical analysis

The linear regression was analyzed using Statistix V8.0.

Results and Discussion

Table 1: Bacterial counts (log CFU/mL) of bulk milk sample clas sified according to methylene blue reduction (TBC=total bacterial count; mean±SD (n))

MB	TBC	*Staphylococcus aureus*	*Streptococcu* spp.	Coliform
> 6hr.	4.98±0.685(54)*	4.03±0.689(2)	4.09±0.876(20)	3.96 0.779(25)*
4-6 hr.	5.63±0.855(219)**	4.85±0.887(5)	4.07±1.255(74)	4.40±0.868(102)**
<4 hr.	6.44±0.581(87)***	4.50±0.750(7)	4.82±1.390(39)*	5.28±0.723(48)***
Total	**5.73±0.898(360)**	**4.56±0.784(14)**	**4.29±1.285(133)**	**4.58±0.934(175)**

*significant difference (p<0.05)

There is statistical significance in the linear regression relationship between total bacterial counts and both dye reduction tests (MB; R^2=0.295 and RR; R^2=0.111). Lactic acid production and pH determination are related to TBC, MB and RR tests. However, the acidity and pH are able to show a significant relation to TBC after 4 and 2 hrs of incubation, respectively. The difference and diversity of the microorganisms may play an important role in acid production which affects the pH of raw milk. Acid titration may not be suitable for detecting pH change due to microorganism contamination in the raw milk sample. A further development of a more sensitive direct or indirect method that is able to detect the level of microorganism contamination in raw milk is still in progress.

Acknowledgment

We appreciate the research fund provided by the Faculty of Veterinary Science, Chulalongkorn University

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P11 Characteristics of Bucket Type Milking Machine Pulsation in Small Dairy Holders

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Introduction and Objective

Milking machine performance affects the udder health of dairy cows. The majority of Thai small dairy holders have used bucket type milking machines for several years. Regular examination of milking machine performance is a crucial procedure for the mastitis control program.

Suboptimal performance of milking machines leads to mastitis which is the most costly disease of dairy cattle.

Our study investigates milking machine performance in small dairy holders of Nakhonpathom province.

Materials and Methods

Twenty eight small dairy holders in Nakhonpathom province were investigated where thirty three bucket type milking machines were examined using a Pulsatortestor Pt V(tm)

Results and Discussion

The vacuum, pulsation, milking phase, rest phase and limping characteristics of the milking machines were determined before afternoon milking. The results are shown in table 1.

There were four categories of abnormalities or suboptimum milking machine performances.

First, 30.3% of the machines had a low vacuum level which might have been due to pump size and pipe line length. Second, 69.7% of machines had a high pulsation rate which might have been due to a compensation of low vacuum. Third, there was some degree of abnormal milking and rest phases. Finally, almost half of the machines showed a limping in which left and right udder could not be emptied at the same time.

Table 1 Characteristics of pulsator performance

	Range	**% (n = 33)**
Vacuum	Lower (0-45 kPa)	30.30
(45-55 kPa)	Normal range (45-55 kPa)	60.60
	Upper (55-65 kPa)	9.10
Pulsation rate	Lower (0-57 t/m)	15.15
(60 t/m)	Normal range (58-62 t/m)	15.15
	Upper (62-130 t/m)	69.70
A+B	Lower (0-57%)	0.00
(60%)	Normal range (58-62%)	81.82
	Upper (63-85%)	18.18
C+D	Lower (0-37%)	15.15
(40%)	Normal range (38-42%)	75.75
	Upper (43-50%)	9.10
B	Lower (0-27%)	0.00
(30%)	Normal range (28-32%)	6.06
	Upper (33-70%)	93.94
D	Lower (0-13%)	3.03
(15%)	Normal range (14-16%)	0.00
	Upper (17-35%)	96.97
Limping	Normal range (0.0-1.0%)	51.52
(1%)	Upper (>1.0 %)	48.48

Pulsator abnormalities of milking machines can be a predisposing cause of mastitis.

The malfunctioning pulsator such as 1) high pulsator rate can cause teat end damage and an increase the rate of new infection. 2) limping can cause incomplete milk removal from udder in which post at risk of mastitis.

Abnormalities in milking machine performances are one of the major predisposing causes of mastitis. Pulsator should be regularly examined, cleaned and maintained (every month) in a mastitis control program.

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P12 3M Petrifilm™ Aerobic Count Plate and *E. coli*/Coliform Count Plate for Enumeration of Bacteria Associated with the Methylene Blue Reduction Test in Raw Milk

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Introduction and Objective

The microbiological quality of raw milk is one of the most worrying food safety concerns of milk. The methylene blue reduction test (MBT) is usually conducted in order to determine the microbiology of raw milk in Thailand.

This study evaluates the correlation between the bacterial enumeration of raw milk using 3M Petrifilm™ and the methylene blue reduction test.

Materials and Methods

A total of 511 raw milk samples from small holder dairy farms of the milk collecting Center in Prachuapkhirikhun (128), Saraburi (159), Chiang Mai (99) and Nakhonpathom (125) provinces during December 2005 to September 2006. All samples were analyzed for MBT and their total bacterial count or TBC (3M Petrifilm™ Aerobic Count Plate) and Coliform Count or CC (3M PetrifilmTM *E. coli*/ Coliform Count Plate). Regression analysis of geometric mean of log10 bacterial count and methylene blue reduction times were determined using Statistic® version 8.

Results and Discussion

Mean of methylene blue reduction time of fresh raw milk collected from farms in Prachuapkhirikhun, Saraburi, Chiang Mai and Nakhonpathom provinces were 5.97, 5.42, 5.88 and 4.94 hours respectively. There were significant differences on TBC among four provinces.

The regression relationship between the log10 TBC and the reduction time of samples from Prachuapkhirikhun, Saraburi, Chiang Mai and Nakhonpathom provinces and in overview were statistically significant when considering the increased reduction time for 1 hour determining that TBC would be decreasing as 0.19, 0.39, 0.34, 0.40 and 0.30 log10 CFU/mL but sample size were less than 50% in each group (R^2 = 0.13, 0.49, 0.31, 0.48 and 0.29 respectively) indicating a relationship between the two tests. Most samples had high reduction times and had a high bacterial cell count. Therefore MBT is not a suitable test for the microbiological quality assurance in raw milk.

The relationship between MBT and CC (R^2 = 0.04, 0.24, 0.11, 0.27 and 0.10 respectively) indicated most samples had a good reduction time while some had numerous coliform bacteria in contaminated raw milk.

The relationship of MBT and CC was statistically significant when samples had increasing reduction time of 1 hour so CC would be decreased for 0.09, 0.30, 0.24, 0.37 and 0.21 log10 CFU/mL. But these results originated from milk samples less than 30%.

Both 3M Petrifilm™ for the enumeration of TBC and CC in raw milk could be using as well as the agar plate method therefore it should be applied to milk quality because they are simple and inexpensive methods compared with conventional methods. Furthermore, there are several factors that affect the reduction of MBT activity such as light, oxygen, bacterial species, leukocyte, fat, dye concentration, incubated temperature and inversion during incubation.

MBT may not be accurately used to estimate bacterial enumeration in raw milk.

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P13 Effect of Polysaccharide Gel from Durian Rinds as a Postmilking Teat Dip Under Experimental Challenge With *Staphylococcus Aureus*

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Introduction and Objective

Mastitis is not only a great cause of economic losses but also a serious public health problem. *Staphylococcus aureus*, a major contagious mastitis pathogen, can be prevented by effective teat antisepsis. Preparations of natural products are alternative remedies since chemical agents will be restricted shortly.

Interestingly, polysaccharide gel (PG) extract from durian rinds has antibacterial properties against most of the mastitis bacterial isolates and also has gelling properties that are suitable for gel formulation as a teat dip sanitizer. This study was to investigate the bactericidal effect of PG teat dip prepared from durian rinds against *S. aureus* using an experimental challenge protocol.

Materials and Methods

The PG was extracted from the dried fruit-rind of durian with boiling water. The PG was precipitated by acidified ethanol, filtered, dried and ground. The teat dip containing 2.5% PG (w/v) in 1% Ringer's solution with plasticizers including 10% glycerin and 10% propylene glycol were prepared.

The teat dip evaluation procedure was carried out according to the National Mastitis Council. In brief, the 44 quarters eligible for new intramammary infections (IMI) from 11 cows were used for controlling the infectious trial. Each teat of a tested cow was immersed in a suspension containing *S. aureus* ($5x10^7$cfu/ml) after the milking machine had been removed at afternoon milking. After challenge, two contralateral teats were dipped with the tested PG teat dip and the remaining two teats were undipped controls. The number of new IMI in quarters of tested groups was investigated.

Results and Discussion

The inhibitory efficacy of PG teat dip prepared on new IMI is summarized in Table 1. The PG teat dip showed a marked reduction in the number of new IMI caused by *S. aureus* at 100% ($p < 0.05$). In total, the new IMI number was 0 (0%) and 5 (22.73%) in quarters treated with the PG teat dip and the un-dipped control group, respectively. Skin irritation was not observed during the study period.

Table 1. Summary of the evaluation of the PG teat dip on new IMI against *S. aureus*

	Control Group	PG teat group
Quarters eligible for new IMI	22	22
New IMI	5	0
Quarter infection rate (%)	22.73	0
Quarter day at risk	492	505
New IMI per 100 quarter day at risk	1.02	0
Reduction rate (%)	100*	

*Significant difference between groups ($p < 0.05$)

The PG teat dip has a benefit for both mastitis prevention and the protection of consumers from drug residues in milk. In addition, PG has not found to induce toxicity in laboratory animals has been well reported. Therefore it is expected to be a promising teat dip for mastitis control.

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P14 Dorsal Patellar Luxation Management in the Beef Cow

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Introduction and Objective

Dorsal patellar luxation of cattle can cause abnormality of gait (jerky gait) due to the abnormal function of the tibia-femoral-patellar articulation. The displacement of patellar always occurs in the dorsal position. The cause of this problem is uncertain, for example, trauma from hard work, malnutrition at pregnancy and post calving period, poor body condition, poor feed quality, post-legged conformation, etc. Cattle in need of dorsal patellar luxation will show chronic lameness. The purpose of this paper is to report the outcome following the use of the surgical technique so called medial patellar desmotomy for correction of dorsal patellar luxation in both hind legs of a beef cow.

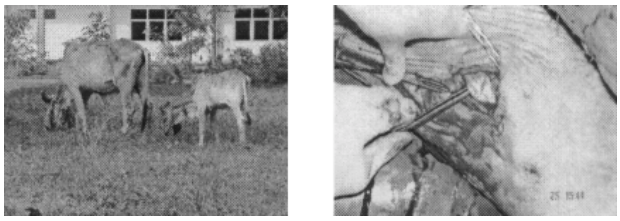
Materials and Method

A six-year old Brahman cow which had shown abnormal gait of both hind limbs for 5 months after parturition was referred to Kasetsart Kamphaengsaen Veterinary Teaching Hospital The body condition score was poor (2 from 9 scales). Inspection with palpation the patellar when the cow walked, weaked unusual patellar movement. Lameness examination also allowed us to see a catch at the end of stifle extension and at the beginning of flexion calling "jerky gait", the typical gait of the dorsal patellar luxation. The treatment plan was medial patellar ligament desmotomy of both hind limbs.

The medial patellar ligament can be located by using the thumb placed at the tibial tuberosity and the middle finger placed at the upper spot of femoral medial trocheal crest. The area between those two fingers is medial patellar ligament.

The cow was sedated for lateral recumbency with Xylazine hydrochloride 0.1 mg/kg bw (intravenously). An aseptic technique at surgical site was prepared. Local anesthesia with 2% of lidocaine hydrochloride was performed at the incision line above the medial patellar ligament. The site of medial patellar ligament desmotomy was close to the insertion part at tibial tuberosity. The cutting space was around 0.5cm. The suturing of subcutaneous and skin was done in the usual manner.

Results and Discussion



A **B**

Fig 1 Picture of the Brahman cow and its calf after medial patellar ligament desmotomy (A), and the medial patellar ligament at the medial stifle position (B).

After the recovery period, the cow could easily stand and walk normally without any complications. The surgical wound had no complications. The prognosis for this case is generally good to excellent. However, if we insufficiently cut the medial patellar ligament, a connection from the fibrous band between the cutting edges could happen allowing the upward fixation of the patellar again in the future.

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P15 Study of the Correlation between the Enzyme Activity of Glucose-6-Phosphate Dehydrogenase and the Hematological Parameters of Stored Whole Blood in Dogs

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Introduction and Objective

Erythrocytes require a reduced form of glutathione, which is regulated by glucose-6-phosphate dehydrogenase (G6PD), a key enzyme in the pentose phosphate pathway (PPP) to maintain shape and membrane stability (1). The objective of the present study was to determine the correlation between the activity of G6PD and the hematological parameters in stored whole blood using CPDA-1 in dogs.

Materials and Methods

Blood was collected from 20 healthy dogs and stored in CPDA-1 solution, at 4-6°C. Changes in the activity of G6PD and hematological parameters were analyzed weekly over 4 wk of storage. The differences between the values at wk 0 and values over consecutive weeks were compared using One Way Repeated Measures ANOVA. The correlation between the activity of G6PD and hematological parameters was performed using Pearson Product Moment Correlation. The statistically significant difference was defined as $p < 0.05$.

Results and Discussion

There were significant decreases in RBC count, Hb concentration, PCV, and MCV, and significant increases in MCH and MCHC when compared to the values at wk 0 after a period of 1 wk of storage. The activity of G6PD during storage was variable and was not significantly different from wk 0. The results are presented in Fig. 1. No correlation between the activity of G6PD and hematological parameters was found in this study.

The present study demonstrated that stored whole blood in CPDA-1 was hemolyzed and erythrocytes were microcytic, hyperchromic during the 4 wks of storage. G6PD did not play a role in the hemolysis of erythrocytes. This study showed that whole blood of dogs could be stored in CPDA-1 solution for a period of at least 4 wks after blood collection.

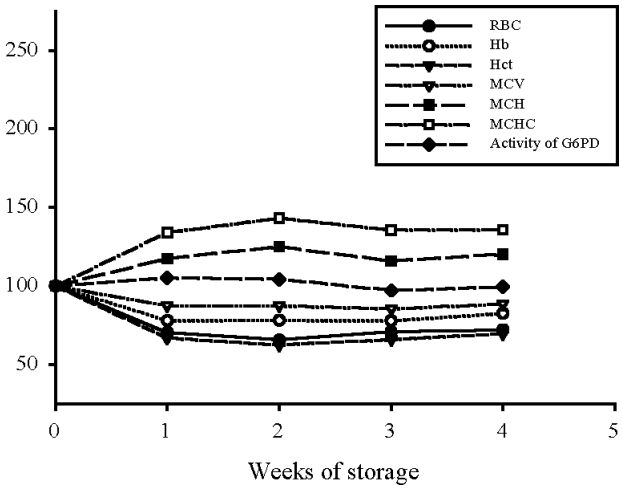

Fig. 1 Percentage changes in RBC, Hb, PCV, MCV, MCH, MCHC, and enzyme activity of G6PD of whole blood during the period of storage

Acknowledgement

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P16 The Correlation between the Progesterone Receptor and the Proliferative Marker, Ki-67 in the Bitch Uterus at Different Stages of the Oestrous Cycle

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Introduction and Objective

One of the most important effects of steroid hormones in reproductive organs is proliferative activity. (1,2,3). In order to further knowledge about the correlation of progesterone and its receptor with uterine proliferation, the objectives of the present study were to provide an immunohistochemical evaluation of the progesterone receptor (PR) and of the Ki-67 proliferative marker, and to determine the relationship between these two proteins in the bitch uterus at different stages of the oestrous cycle.

Material and Methods

The uterine horns were classified into 4 groups according to the oestrous stages; these were prooestrus (n=3), oestrus (n=3), dioestrus (n=3) and anoestrus (n=3).

The presence of PR and Ki-67 was investigated by immunohistochemistry using mouse monoclonal antibodies. The results of the PR total score and the Ki-67 labelling index was also correlated.

Results

The highest PR and Ki-67 immunostaining score was found at oestrus in all compartments. However, the Ki-67 labelling index was significantly highest only in the surface epithelium at prooestrus and oestrus.

A significant correlation was observed between PR positive and Ki-67 positive cells in the surface epithelium.

Discussion

The results from the present study strengthen the finding that the presence of PR and uterine proliferation were different during the stages of the oestrous cycle and among different uterine compartments. The positive correlation between PR and Ki-67 may indicate the same regulator of these two proteins in the surface epithelium and may suggest the role of PR in proliferative effects in addition to oestrogen and its receptor, at least in the surface epithelium of the bitch uterus.

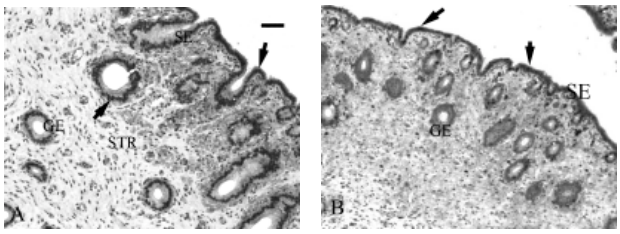

Fig A PR immunostaining at oestrus; Fig B, Ki-67 immunostaining at prooestrus. SE = surface epithelium; GE = glandular epithelium, STR = stroma and M= myometrium. The arrows show positive staining cells and the bar in Figure A represents a distance of 50 μm.

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P17 Expression of the GPIIb/IIIa Receptor on Canine Platelets with Naturally Occurring Monocytic Ehrlichiosis: Pre- and Posttreatment with 6-weeks of Doxycycline

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Introduction and Objective

Canine monocytic ehrlichiosis (CME) is a tick born disease caused by the *Ehrlichia canis*. Thrombocytopenia is the most prominent and consistent hematological change. Dogs that recover from severe thrombocytopenia still show bleeding tendencies, which suggest that platelet dysfunction is present (1). GPIIb/IIIa or CD41/CD61 complex, a glycoprotein platelet surface receptor, plays important role in platelet aggregation and clot retraction (3). The aim of this study was to evaluate the expression of the CD41/CD61 complex in naturally occurring CME before and after 6 weeks of doxycycline treatment.

Materials and Methods

Blood samples from 8 dogs that had been naturally infected with *Ehrlichia canis*., the presence of morulae of *Ehrlichia canis* in the cytoplasm of mononuclear cell and positive of ELISA kits test were selected for this study. Doxycycline (10 mg/kg as a loading dose and then 5 mg/kg PO SID for 6 weeks) was prescribed for every dog. Blood samples from each dog were made pre-and post treatment. Changes in the specific markers of platelet activation by use of adenosine diphosphate was determined (2). Cell staining was performed using antihuman CD41- and CD61-FITC. Measurement of platelet activation with CD41/61 expression was performed using a FACSCalibur flow cytometer with MacIntosh CellQuest software. Platelets were identified and gated by their characteristic forward and side scatter. Changes in surface specific markers of platelet activation in pre- and post treatment were compared.

Results and Discussion

Flow cytometric assay demonstrated that that expression of CD41 and CD61 of the pretreatment samples (63.8%±3.5% and 78%±6.5%) were lower than postreatment samples (86.5%±7.2 %)% and 89%%±5.3%). The results suggest that low GPIIb/IIIa expression may be a contributing factor to platelet dysfunction in CME. These findings suggest that 6 weeks of doxycycline treatment may help CME dogs recover from platelet dysfunction.

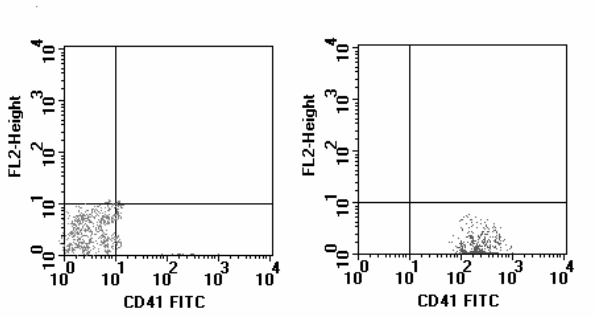

Fig 1. Flow cytometric analysis of the antigen recognized by CD41 in pre- (left) vs. posttreatment (right).

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P18 Development of *Brugia pahangi* in Experimental Vector, *Aedes aegypti* (Liverpool strain)

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Introduction and Objective

The efficiency of *Aedes aegypti* and the ability of *Brugia pahangi* to produce a number of L3 may serve as an important factor enhancing the spread of the disease. This study is to observe the complete developmental biology of the filarial worm, *B. pahangi* in the experimental mosquito, *Ae. aegypti* (Liverpool strain). Inspite of the fact that filarial rate of *B. pahangi* among stray cats around Bangkok has dropped from 25.3% to 10% in the past decades, the rate in dogs has increased from 0 to 4.17% as seen in the recent records (1). We therefore recorded the morphology of *B. pahangi* (L1 through L3) and the time coverage in days in the female experiment mosquito. The complete life cycle of *B. pahangi* in the insect is compared to other natural vectors such as *Ae. togoi* and *Anopheles quandrimaculatus.*

Materials and Method

Brugia pahangi, was confirmed by using the enzyme acid phosphatase activity on microfilaria, naturally infected cats. The *Ae. Aegypti* (Liverpool strain) used in the experiments had been maintained in Parasitology Unit, Department of Pathology, Faculty of Chulalongkorn University since 1999. Five day old female mosquitoes were starved for about 12 hours prior to feeding. Usually the feeding was carried out in the afternoon. The infected mosquitoes were dissected daily from 1-14 days for the development of filarial larvae. Each mosquito was separated into head, thorax and abdomen using dissecting needles and placed in a different drop of normal saline on the slide. Each part was teased apart and examined carefully under the light microscope.

Results and Discussion

On dissection of engorged mosquitoes after 24 hrs, the ingested microfilariae were found in the thoracic muscles. The microfilaria became shorter and more tumpy than microfilaria known to the first stage larva or sausage form. The first molt occurred between 4-5 days found in the thoracic muscle. Then larvae became longer in length with rapid cell multiplication and the intestinal organs became developed. The larva moved sluggishly when removed from the thoracic muscles. This stage was known to be the second stage. After 8 days, the larvae had either completed the second molt or were still in the process of molting then becoming the third stage larva. After 10-14 days, third stage larvae moved very actively and they could be found mainly in the mouth parts (Fig 1). L3 was also found in the head portion, in thoracic muscles as well as in the abdomen of *Ae. aegypti*.

In this study, the development pattern of *B. pahangi* in the experimental vector was similar to *Anopheles quandrimaculatus* and *Ae. togoi* (2).

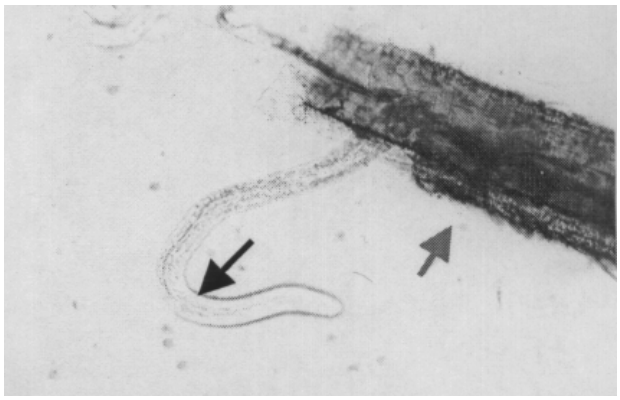

Fig 1. The third developmental stage larva (L3) (black arrow) in proboscis (red arrow) of *Ae. aegypti* is found 10-14 days after infection.

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P19 The Effect of Anesthetic Drug on Microfilariae Density of *Brugia pahangi* in Cats

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Introduction and Objective

The extensive study of filariasis of both human and animals regarding their pathogenesis, immune response, epidemiology and parasite-vector relationship have mostly been conducted in the animal model. For instance, cats have been used as the model for *Brugia pahangi* to determine the filarial rate and density as previously described (1). The effects of anesthetic drugs applied during the experiments are of serious concern. The level of parasitemia (microfilaremia) may be altered when animals are anesthetized. We therefore compared the level of microfilaremia before and after the application of xylazine/ ketamine in cats with microfilaria of *B. pahangi* in the circulation. This study may help eliminate the interference of anesthetic drug during the course of experiment.

Materials and Method

Fourteen cats were naturally infected with only *B. pahangi* confirmed by acid phosphatase staining technique. They were injected with an anesthetic drug, then blood samples were obtained from the femoral vein in order to determine the circulating microfilaremia by three line thick smear and Giemsa's staining techniques. The anesthetics used were atropine sulphate (0.04 mg/kg), xylazine (1 mg/kg), and ketamine (10 mg/kg) injected intramuscularly. All studies were conducted between 4 pm and 7 pm. Twenty minutes after the animals became anesthetized, blood sample was taken and the circulating microfilaremia were counted. Data was analyzed using Pair-T test to compare the microfilaremias of the pre- and post anesthetic conditions.

The numbers of microfilariae in the 14 cats after anesthetization increased significantly ($p < 0.01$) (Table 1). According to the previous report, there had been an effect of anesthetics on the peripheral blood microfilaremia of *B. malayi* in Mongolian jird, *Meriones unguiculatus* (2). This suggested the increased microfilaremia was found when jirds received Rumpun and Ketaset. This is in the line with our study. However, jirds injected with sodium pentobarbital did not increase the microfilaremia. Our data suggests that increases in microfilaremia may be induced by xylazine or ketamine or both. However, xylazine/and ketmine are routinely recommended for anesthetization in cats rather than sodium pentobarbital.

Results and Discussion

Table.1 The numbers of microfilaremia of *B. pahangi* infected cats, determined before and after anesthetic condition.

Cat No.	before (mf/1ml)	after (mf/1ml)
1	7,800	9,300
2	3,683	6,033
3	4,183	8,083
4	767	883
5	667	683
6	2,417	4,267
7	2,017	7,933
8	2,183	2,450
9	8,233	11,500
10	4,300	10,883
11	2,700	3,600
12	11,050	17,367
13	1,967	2,067
14	9,600	10,200

Acknowledgment

We would like to thanks Assist. Prof. Dr. Padet Tummaruk for statistic analysis.

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P20 Genetic Parameters on Growth Traits (1) : Variance Components and Heritabilities

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Introduction and Objective

The potential for genetic improvement depends on genetic parameters which are especially needed to provide quality breeding stock. The objective was to estimate variance components and heritability for weaning weight (WW), final weight (FW) and average daily gain (ADG) of New Zealand White rabbit.

Material and Methods

Data on doe growth traits were collected over a 10-year period (1995-2005) from the Veterinary Student Training Center of Chulalongkorn University. An animal model was employed to estimate genetic δ_a^2), environmental δ_e^2) and total δ_p^2) variances for growth traits by AIREML (average information restricted maximum likelihood algorithm) (7,8). The animal model terms included the fixed effect of year-month of birth, sex, parity, litter size, fattening date as covariate effect, random doe (additive genetic) and residual effects.

Results and Discussions

The additive genetic variances (Table 1) indicated a strong contribution of additive genes in WW and FW traits, but the ADG was low because of no restricted fattening date during post-weaning to the market period. The heritability and standard error (h^2±SE) of WW was higher (9) perhaps because of breed characteristics, method calculation and environmental differences. The FW was moderate (3). It may be the small size of progeny/ generation (1,4). The ADG was less than other reports (2,5,6,9) which were ranged from 0.13 to 0.48. This was due to a low additive variance but the error variance was so high.

Table 1 Genetic parameters of growth traits

Traits	Genetic parameters			
	δ_a^2	δ_e^2	δ_p^2	h^2±SE
WW	18,422	18,469	36,890	0.500±0.006
FW	21,166	42,128	63,293	0.334±0.009
ADG	2.8482	51.22	4.07	0.053±0.08

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P21 Genetic Parameters on Growth Traits (2) : Phenotypic and Genetic Correlations

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Introduction and Objective

The most useful correlation in animal breeding for the determination of selection criteria is a correlation between two traits in a population. Phenotypic correlation measures the relationship between two performance traits and genetic correlation shows the relationship between their breeding values.

$$y = X\beta + Za + e$$

Material and Methods

Data were collected over a 10-year period (1995-2005) from the Veterinary Student Training Center of Chulalongkorn University. The following animal model was used

where y is a vector of observations on the animal, is a vector of fixed effects of year-month of birth (1 to 154), sex, parity (1 to 17), litter size (1 to 13), fattening date as covariate effect. a is a random doe (additive genetic) and e is a residual effect. The model was employed to estimate (co)variance components in order to calculate phenotypic and genetic correlations (2) between growth traits by AIREML (average information restricted maximum likelihood algorithm) (3,4).

Results and Discussions

In Table1, moderate phenotypic and genetic correlation between WW and FW are positive (1). The result indicates that selection for WW would give a desirable increase in FW.

Table1 Phenotypic (lower) and genetic (upper diagonal) correlations for weaning weight (WW), final weight (FW) and average daily gain (ADG) of New Zealand White rabbits

Traits	WW	FW	ADG
WW	-	0.560±0.006	-0.339±0.01
FW	0.420±0.003	-	0.323±0.01
ADG	-0.148±0.060	0.220±0.010	-

In fact, the WW and ADG did not simultaneously occur in the rabbit's lifetime. Correlation between WW and ADG can not be used for selection because it is futile to improve both of them together. Between the FW and ADG, if the FW were higher, the difference between the FW and WW or weight gain will increase and ADG will also increase. These results indicate that when rabbits are selected for increased ADG, FW will be also increased.

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P22 Growth Performances of New Zealand White Rabbits in the Veterinary Student Training Center, Chulalongkorn University, Nakorn Prathom province

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Introduction and Objective

Growth performance is the most importance economic trait because it is related to the benefits of production. Thus, the objective was to study the growth performance in areas such as weaning weight (WW), final weight (FW) and average daily gain (ADG) of New Zealand White rabbits in Veterinary Student Training Center, Chulalongkorn University, Nakorn Prathom province.

Material and Methods

Data on 15,239 New Zealand White rabbits were collected from the local population at Nakorn Pathom province in Thailand between 1995 and 2005. The procedure GLM in the statistical computer8 was employed to identify important fixed effects to be included in the final model. The model was as follows;

$$y_{ijklm} = \mu + YM_i + S_j + P_k + LS_l + bFD_{ijklm} + e_{ijklm}$$

Where y is observation on the m^{th} rabbit of fattening days on the i^{th} year-month, the j^{th} sire, the k^{th} parity and the l^{th} litter size. μ is overall means, *YM* is fixed combined effect of birth year-month (i = 1 to 154), *S* is fixed effects of sex (j =1 to 2), P is fixed effects of parity number (k = 1 to 17), *LS* is fixed effects of litter size (l = 1 to 13), *FD* is partial regression of the observation on fattening days and *e* is a random residual effect.

Results and Discussions

In Table 1, the result of WW in this study was a higher means and standard error than previous reports (1,2,4) because of breed difference. They used synthetic or crossbred rabbit lines which differ from New Zealand White rabbits. The FW were lower in means of FW than Gondret *et al* (2005) (2). This difference could be the result of post-weaning periods which were not equal in each situation. The ADG were less than previous published research (3,4). Otherwise, some research (5,6) reported many more means of ADG than this study which ranged from approximately 40 - 50 g/d. The reason for this problem is that the rabbit population of this study over the past 10 years ago had no selection for improving growth rate like many researches.

Table 1 Numbers of records, least-square means (LSM), standard error (SE) and range for growth performance traits

Traits	Numbers (Records)	LSM (S.E.)	Min	Max
WW (g)	14,983	764.64(229.78)	350	1,420
FW (g)	12,437	1,835.47(405.70)	1,200	3,100
ADG (g/d)	12,412	19.94(9.17)	7.333	6.43

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P23 Laboratory Identification of Streptococcal Bacteria in Cultured Tilapia *(Oreochromis niloticus)*

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Introduction and Objective

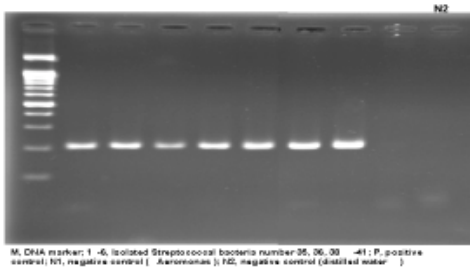
Tilapia *(Oreochromis niloticus)* is being increasingly cultured for food fish in many countries (4). Amongs diseases in intensive tilapia farming, *Streptococcosis* causes the most reduction in stocking of tilapia and has economic consequences on fisheries in many areas of the world. It has been found that there is a greater incidence of Streptococcal infections in any tilapia than in other cultured fish (1,2). Streptococcosis is a septicemia disease, caused by both alpha and beta-hemolytic strains of *Streptococcus* spp. *Streptococcus iniae* and *Streptococcus agalactiae* are the common isolates in fish infections (1,5). Fish Streptococcosis caused by *Streptococcus iniae* is a zoonotic disease, which has been reported in humans with a history of accidental injuries during the handling of fish (3). With the intensive growth of tilapia culture Streptococcal infections are becoming a major threat to the tilapia industry worldwide. The present study aims to identify Streptococcal bacteria in Thai cultured tilapia.

Material and Methods

Diseased fish obtained from clinical cases were used for the study. Bacteria was isolated from the kidneys of diseased fish on TSA agar containing 5% sheep blood. The isolates were cultured at 33°C for 24 hr. Pure cultures were preliminarily examined for bacterial morphology, gram's stain and catalase activity. Biochemical analysis of the bacterial isolates conformed with the API system (BioMeieux, France). Bacterial isolates represented *Streptococcus* spp. morphology and biochemical profiles were further comfirmed by the polymerase chain reaction (PCR). *Streptococcal* DNA was extracted from bacterial cells using the phenol extraction method. Two sets of genus specific oligonucleotide primer; C1 (5'-GCG TGC CTA ATA CAT GCA A-3') and C2 (5'-TAC AAC GCA GGT CCA TCT-3'); were derived from a specific sequence of the 16sRNA gene. The PCR reaction mixture was performed in a final volume of 20 µl containing 250 ng of Streptococcal DNA as template, 2 µl of 10X Tag polymerase buffer (20 mM $MgCl_2$), 2.5 mM each of deoxynucleotide triphosphate (dNTP), 10 µM each primer and 2.5 U Tag DNA polymerase. The reaction was carried out in a PCR thermocycler for 32 cycles; consisting of an initial denaturation at 94°C for 2 min, denaturation at 94°C for 20 sec, annealing at 56°C for 10 sec and extension at 72°C for 30 sec. The last cycle was followed by a final extension step at 72°C for 2 min. Amplified products were separated by electrophoresis in 2% agarose gel and visualized by UV transillumination following ethidium bromide staining. *Aeromonas hydrophila* ATCC 35654 and distilled water were applied as a negative control while *Streptococcus iniae* ATCC 29178 was used as a positive control.

Results and Discussion

The diseased fish was found to have gross lesions as generally described in fish Streptococcosis; including generalized hemorrhage of the visceral organs, serosanguneous peritonitis and congestion of the liver, kidneys and spleen. Bacteriological procedures on the sample obtained from nephrotic tissue showed isolates of dull-white pin point colonies with alpha hemolysis. Microscopic examination of isolates revealed gram positive, long chain cocci. Biochemical analysis and catalase negative observed from all isolates (n = 60) suggested the characteristic similar to *Streptococcus* spp. Confirmative identification using PCR presented the 207 bp PCR products of the 16sRNA gene. DNA amplification of the target gene observed in all isolates correlated to the amplicon obtained from the positive control, *Streptococcus iniae* ATCC29178. The present study has demonstrated bacteriological methods for the identification of fish *Streptococcosis*, the conventional morphological and biochemical technique and DNA based technique.



M, DNA marker; 1 -6, isolated Streptococcal bacteria number 35, 36, 38 ,41 ; P, positive control; N1, negative control (Aeromonas); N2, negative control (distilled water)

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P24 Cloning and Characterization of a Novel Immune-type Receptor Gene, the Immunoglobulin Superfamily (IgSF), from the Japanese Flounder *(Paralichthys olivaceus)*

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Introduction and Objective

The Japanese flounder *(Paralichthys olivaceus)*, a large marine flat fish, is one of the most important fishes in East Asia. Culture of the fish has been developed rapidly. An understanding of the fish's immune system is, therefore, a subject of interest to encourage good quality and quantity. However, information about fish immune systems is still lacking compared to that of mammals. Studies of the Japanese flounder's novel immune-type receptor (poNITR), one of immunoglobulin superfamily (IgSF) receptors, were aimed to elucidate the basic information of immune-type receptors for a further understanding of the fish immune system in terms of function, protein interaction and evolutional relationship.

Materials and Methods

poNITR cDNA and genes were isolated from cDNA and BAC libraries, respectively. The expression of poNITR from many tissues was investigated using RT-PCR. Peripheral blood leukocyte (PBL) was isolated and stimulated with poly I:C and LPS for 1, 3, 6, 12 and 24 h. RT-PCR was performed to detect the gene expression. To detect cell distribution, *in situ* hybridization was carried out in PBL using RNA-labeled probes of NITR, IgM and TCR-α.

Results and Discussion

poNITR cDNA encoded 357 amino acid residues. The amino acid sequence identities between poNITR and previously reported NITRs were approximately 30%-40%. The poNITR consists of two immunoglobulin (V and V/C2) domains in the extracellular portion, a transmembrane and immunoreceptor tyrosine-based inhibitor motifs (ITIMs) in the intracellular portion. The mixed features of NITRs are believed to be a representation of an integral stage in the evolution of innate and adaptive immune receptors. The poNITR gene is composed of five exons and four introns spanning approximately 3.4 kb. The poNITR transcript was mainly detected in the gills, head kidney, trunk kidney and intestines and was slightly detected in the heart, muscle, peripheral blood leukocytes, skin, spleen and stomach. However, the poNITR gene expression was not detected in muscles and ovaries. The poNITR gene expression was not induced by LPS and poly I:C. Based on these results, it is indicated that the expression of NITRs may not be stimulated by viruses or bacteria. *In situ* hybridization reveals that the poNITR was expressed in both TCR-α- and IgM-presenting cells.

P25 Antimicrobial Activity of Aqueous and Ethanol extracted Indian Almond Leaves (*Terminalia catappa* Linn.)

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Introduction and Objective

The indian almond tree (*Terminalia catappa* Linn.) is a Combretaceous plant (tropical almond family), which can be found throughout Thailand. The various extracts of leaves and bark of the plant have been pharmacologically studied and reported to have anticancer, antioxidation, anti-HIV reverse transcriptase, hepatoprotection, anti-inflammation, aphrodisiac activities, antifungal properties againt *Pythium ultimum, Rhizoctonia solani, Sclerotium rolfsii,* and *Aspergillus fumigatus,* and antibacterial action against; *Staphylococcus epidermidis, S.aureus, Bacillus cereus, B. subtilis* and *Pseudomonas aeruginosa*. The chemical composition of this plant includes tannins, flavanoids, isovitexin, vitexin, isoorientin, rutin and triterpenoiods (3). The objective of this study is to evaluate the antibacterial activity of water and ethanol extraction of dried Indian almond leaf.

Material and Methods

Plant Material and preparation of extracts

The leaves of *Terminalia catappa* Linn. were collected in Bangkok, Thailand. Dried leaves were cut in to small pieces. Maceration in twenty liter of distilled water and 95% ethanol for 7 days at room temperature. The extracted fluid was filtered by a Whatman filter paper No. 4 (2). Both extracts were dried at 50°C in a vacuum till solid to semisolid, and then stored in sterile containers in a refrigerator (4°C) until used.

Antibacterial assay

The antibacterial activities of the extracts were determined using the Agar dilution method. The bacteria isolates used in the study were derived from fish and other aquatic animal from the Veterinary Medicine Aquatic Reserch Center (VMARC), Chulalongkorn University, Thailand. The isolates consisted of: *Aeromonas hydrophila, A. sobria, Photobacterium damsela, Pasteurella pheumotropica, Burkholderia cepacia, P. aeruginosa, P. oryzihabitans, Proteus valgaris, Vibrio parahemolyticus, V. fluvialis, V. alginolytica, Shewanella putrefaciens, Stenotrophomonas maltophilia, Klebsiella pneumoniae* and *Enterococcus fecalis*. The API-20E test strip (bioMerieux® SA France) was used for bacterial identification.

A. hydrophila , A. sobria, P. aeruginosa, P. damsela, P. valgaris, E. fecalis and P. pheumotropica were cultured on Mueller-Hinton agar plate. Other bacteria isolates were cultured on Mueller-Hinton +1% NaCl agar plates.

The bacterial suspension was diluted (MacFarland nephelometer tube No. 0.5) into 10^4 cfu/ml and spread on the surface of agar medium plates with a mixture of each concentration of aqueous and ethanol extracted dried Indian almond leave by calculating dilution. Both extracts were concentrations used were 5.0, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.8, 0.6, 0.4 and 0.2 mg/ml. The MIC was determined as the lowest dilution which completely prevented microbial growth. The control plate had only distilled water and 95% ethanol. All samples were tested in triplicate.

Tannin analysis

The Powder of aqueous and ethanol dried Indian almond leave extract was prepared in 50 mg/ml in water and ethanol, respectively. Optical density (OD) was measured by Spectrophotometer at 245 nm. wave length. Total tannin concentrations were determined by Colorimetric method (1).

Table 1 Optical density (OD) and tannin of dried Indian almond leave 50 mg/ml in water and ethanol extraction.

Indian almond leave extraction	Extract yield (%)	OD	Tannin (mg/ml)
Control (distill water)	-	0	0
Control (95% ethanol)	-	0	0
Aqueous Ex.	26.0	0.88	14.08
Ethanol Ex	12.9	1.80	28.97

Results and Discussion

Table 1 shows the percentage yield and tannin level of 50 mg/ml of dried Indian almond leaves with aqueous and ethanol extractions. Table 2 shows the results of the antibacterial activity of both extracts. Indian almond leaf extraction presented the broadest spectrum of action against bacteria, inhibiting all of the stains tested with minimum inhibitory concentrations (MICs) ranging from 0.4-2.0 mg/ml. The ethanol extractions showed stronger activity than the aqueous extractions of dried Indian almond leaves. No growth inhibition was observed in the negative control. These results correlate with the total tannin level of both extracts. In conclusion, Indian almond leaf extract can be used as an alternative to chemical antibacterial treatment. This study provides basic knowledge to reduce the use of chemical and antibiotics in fish culture by using traditional medicinal plants, such as the Indian almond leaf.

Table 2 MIC (mg/ml) of Aqueous and methanol extracted of dried In dian almond leaves

Bacterial species	VMARC Code	MIC (mg/ml) Aq. Ex.	Et. Ex.
Gram negative			
Aeromonas hydrophila	Ah001	1.5	0.8
	Ah002	1.5	0.8
	Ah003	2.0	0.8
	Ah004	1.5	0.6
Aeromonas sobria	As001	2.0	0.6
	As002	2.0	0.6
Burkholderia cepacia	Buc001	2.0	0.8
Pseudomonas aeruginosa	Psa001	2.0	1.5
Pseudomonas oryzihabitans	Pso001	2.0	0.8
Pasteurella pheumotropica	Pap001	0.8	0.4
Photobacterium damsela	Phd001	1.0	0.6
	Phd002	1.5	0.6
Proteus valgaris	Prv001	1.5	1.0
Shewanella putrefaciens	Shp001	2.0	0.8
Stenotrophomonas maltophilia	Stm001	2.0	0.6
Vibrio Parahemolyticus	Vp001	2.0	0.8
Vibrio fluvialis	Vf001	2.0	0.6
Vibrio alginolytica	Va001	1.5	0.6
Gram positive			
Enterococcus faecalis	Enf001	1.0	1.0
	Enf002	2.0	2.0
Klebsiella pneumoniae	Klp001	2.0	1.5

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P26 Comparative Studies of Sea Turtle Prostheses Efficiency Comparing Flapped and Non-Flapped Models

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Introduction and Objective

Sea turtles are considered to be endangered species by the International Union for the Conservation of Nature and Natural resources (IUCN) and the Convention on International trade in endangered species of Flora and Fauna (CITES I).

The locomotion concept of sea turtle swimming may be determined by the "up stroke" and "down stroke" movements of the forelimbs. Such movements originate the "power stroke" which creates the powerful and dynamic turtle type of swimming. The aerodynamic and fluid dynamic analysis of the power stroke shows the lift-based and drag-based mechanisms involved in the movements.

Sea turtles with amputated limb have been found regularly due to various causes such as, physical damage from entanglement (ie. Fish net, rope, propeller), wounds from predators (shark, human), severe infection diseases (ie. bacteria, virus, fungus, etc.).

The complications after limb amputation are impaired locomotion, diving ability, and competitive capability for food with others. These turtle are easily attacked by predators. Therefore, these turtles with amputated limb must be kept and fed separately in captivity.

To develop veterinary prostheses for sea turtles to improve the quality of life of sea turtles for conservation purposes.

Materials and Methods

The prostheses were designed to have aeroplane wing-like characteristics. There were 2 designs experimented with, the first one was with a flap, and second one without flap.

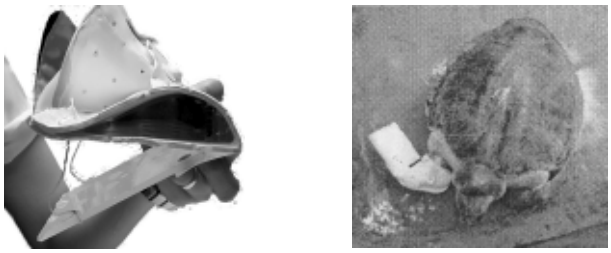

Figure 1 Structure of Prostheses and experimental turtle with flapped prostheses.

Prostheses were designed from limp prostheses in humans and aerodynamics and fluid dynamic theories, They were made from thermoplastic (Thermolyn pedilon®): polyethylene. The prostheses consisted of 3 sections: socket, flipper-flap, and strap.

Two female Olive ridley *(Lepidochelys olivacea)* aged 5 and 10 year old with one forelimb missing were used in the experiments. Both prostheses designs were placed and compared in the same animals. (Fig.1) An efficiency evaluation of movement speed in the turtle was done by computerized video camera which also monitored the behaviour and gestures. Quantitative data were analyzed using a Motion Detection Program. The performance of the prostheses in laboratory experiments was analyzed by Solid work 2005.

Results and Discussion

The result by Motion Detection Program analysis shows that the first turtle had an average speed without prostheses of 74.69±10.30 cm./sec., the average speed of the prostheses with a flap was 99.19±14.38 cm./sec., and the non-flap prostheses had an average speed of 76.90±10.22 cm./sec. The second turtle had an average speed without a prostheses of 42.99±9.91 cm./sec. while the average speed of the flap prostheses was 60.92±7.72 cm./sec and the average speed of the non-flap prostheses was 52.08±12.26 cm./sec.

The performance of the prostheses in the laboratory simulating experiment showed a difference in water velocity measured when flowing over the prostheses. The result indicated that the prostheses with the flap had a higher water lifting force and higher velocity than the one without flap. (Fig. 2)

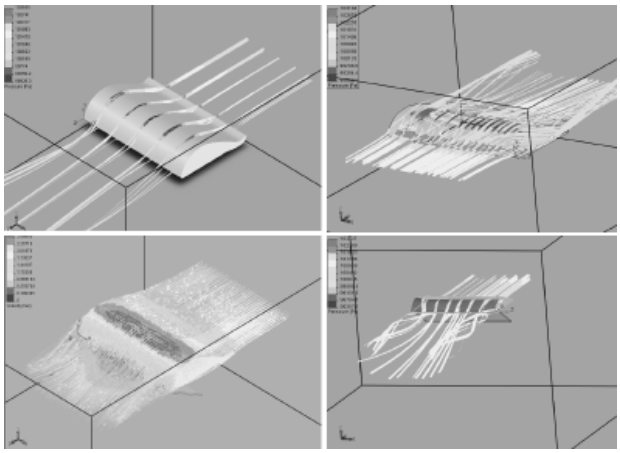

Figure 2 Prostheses analysis by Solid work 2005.

Conclusion

The result indicated that the prostheses with the flap was more efficient for increasing the swimming speed of the turtles than the one without a flap. This study is a pioneer study looking at the feasibility of designing a prostheses for sea turtles which can be developed and improved to assist deformed sea turtles in the future.

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P27 Genetic Analysis of Donkeys and Mules in Thailand Using 5 Microsatellite Markers

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Introduction and Objective

Genetic polymorphisms at DNA-level can be successfully used for population analysis and for establishing relationships within and among species. Few publications describe DNA markers in the donkey.

The objective of this study was carried out to characterize a genetic analysis of donkeys and mules based on allelic frequencies for 5 microsatellite loci.

Materials and Methods

Hair samples from 6 donkeys and 34 mules located in Chiangmai and Chiang Rai provinces were collected. DNA was extracted according to Loftus et al. (1999). Five equine microsatellite primer pairs were used: AHT4 (1), ASB17 (2), HMS6, HMS7 (3) and HTG10 (4). A polymerase chain reaction was performed in a final volume of 8 ul. PCR products were run on ALFexpress II (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Fragment size analysis was performed after the processing of raw data with software ALFwin™ Fragment Analyzer Version 1.03.

Microsatellite allele frequencies, observed (Ho) and expected (He) heterozygosity, polymorphic information content (PIC), and exclusion probabilities (PE) were calculated using the Cervus programme (6).

Results and Discussion

The equine microsatellites were all well-amplified in the donkeys and mules. All amplified loci were polymorphic except ASB17, which was monomorphic (92 bp) in donkeys. The number of alleles in the donkeys varied between 3 (HMS6) and 6 (HTG10) whereas in the mules varied between 8 (AHT4) and 13 (ASB17) (Table 1).

The results showed high polymorphic information content which provided enough information to develop a parentage verification and individual identification system.

References

1. Binn et al., 1995.
2. Breen et al., 1997.
3. Guerin et al., 1994.
4. Loftus et al., 1999.
5. Maklund et al., 1994.
6. Marshall et al., 1998.

Table 1. Range and total number of observed alleles, observed (Ho) and expected (He) heterozygosity, polymorphic information content (PIC), and exclusion probabilities(PE) in donkey and mule

Locus		Size range (bp)	No. of alleles	Ho	He	PIC	PE
AHT4	- Donkey	152-158	4	0.667	0.773	0.683	0.714
	- Mule	148-164	9	0.971	0.858	0.826	0.478
ASB17	- Donkey	92	1	-	-	-	-
	- Mule	92-124	13	1.000	0.867	0.841	0.442
HMS6	- Donkey	155-161	3	0.333	0.667	0.573	0.813
	- Mule	153-169	8	0.971	0.827	0.792	0.531
HMS7	- Donkey	173-179	4	0.833	0.636	0.557	0.818
	- Mule	165-185	10	0.824	0.877	0.849	0.430
HTG10	- Donkey	87-103	6	0.833	0.848	0.762	0.606
	- Mule	87-107	12	0.882	0.895	0.871	0.385

P28 Influence of the Methanolic Extract from *Abutilon indicum* Leaves in Normal and Streptozotocin-Induced Diabetic Rats: Involving α-Glucosidase Inhibition

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Introduction and Objective

The use of plants to treat diabetes has been increase in many countries, including Thailand. One of the medicinal plants that we are interested in in this study is *Abutilon indicum (AI)* or "Krob-Fun-See". Its leaves are traditionally used for the treatment of diabetes, diuretic infection, gingivitis and inflammatory disease. The aim of this study is to evaluate methanolic extract of AI leaves in normal and STZ-induced diabetic rats.

Material and Methods

Normal and diabetic rats were divided into 4 groups with 6 rats in each group. Group 1 was the control group receiving 1% tween. Group 2, 3 and 4 received 250, 500 mg/kg of the extract and 500 mg/kg of metformin, respectively. Blood samples were collected before and after 30, 60 and 120 min following administration. Furthermore, we investigated any *in vivo* and *in vitro* α-glucosidase inhibition activity of the AI extract.

Results and Discussion

The chemical screening of the extract showed the phenolic compound and flavonoid content were 1.04 ± 0.01 mg/g and 59.92 ± 3.88 μg/g extract, respectively. A single oral administration of the extract (500 mg/kg) decreased blood glucose concentrations significantly in both normal and diabetic rats 2 h after administration. Metformin was used as the reference drug and it reduced the blood glucose concentration only in diabetic rats (Fig 1). To clarify the involved mechanism, normal rats were orally administered with sucrose and maltose at dose of 3 g/kg with or without AI extract. The postprandial elevation in the blood glucose concentrations at 30 min following the administration of sucrose with the extract was significantly suppressed when compared with the control group. No significant change in blood glucose concentrations was observed in maltose-loaded rats. An *in vitro* study indicated that *AI* extract inhibited α-glucosidase, a disaccharide-digesting enzyme in the small intestine. The extract showed potent sucrase inhibitory activity with IC50 of 2.45 ± 0.13 mg/ml while the extract was less potent on maltase inhibition. These results suggest that the extract from *A. indicum* leaves would be effective for lowering and suppressing the elevation of postprandrial blood glucose concentrations.

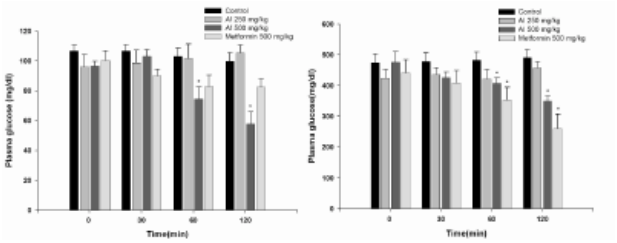

Fig 1. Effect of *AI* leaves extract on blood glucose concentrations in normal rats (A) and STZ-induced diabetic rats (B). Data were expressed as mean ± S.E.M., n =6. *$P < 0.05$.

P29 The Effect of Genistein and Daidzein on Anxiety Levels: In Comparison to Estrogen in Ovariectomized Rat

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Introduction and Objective

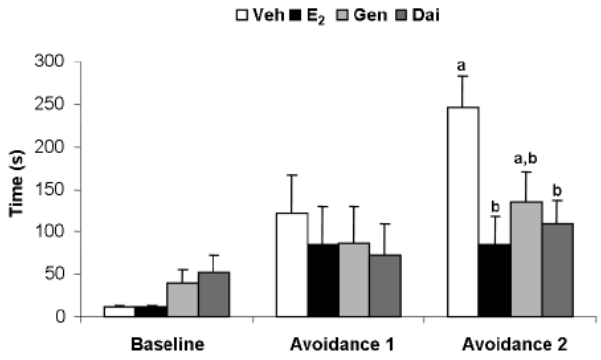
Phytoestrogens (i.e. daidzein, genistein) are plant derived bioactive compounds with a chemical structure similar to 17 β-estradiol (1). Therefore, they can bind to the estrogen receptor and act as receptor agonists or antagonists. It is known that there are 2 types of estrogen receptor, alpha (ERα) and beta (ERβ); in which both daidzein and genistein are preferably bound to ERβ (1). These receptors are unequally distributed in the body; ERα is predominantly located in the reproductive organs while ERβ is found at other sites including the brain. Since lack of estrogen is related to some psychiatric conditions such as anxiety and depression, it can be alleviated with estrogen replacement therapy (ERT) (2). However, in some cases the ERT is prohibited, for instance in hormone-dependent tumors; therefore, phytoestrogens may be an alternative consideration. Herein, we hypothesize that by binding to ERβ, daidzein and genistein is able to reduce anxiety comparably to estrogen, but has less effect on the reproductive organ of ovariectomized rats.

Materials and Methods

Female Wistar rats were ovariectomized and divided into 4 groups: Veh (Vehicle; 10% DMSO in PG), E_2 (Estradiol; 10 μg/kg), Gen (Genistein; 0.5 mg/kg) and Dai (Daidzein; 0.5 mg/kg). Four weeks after ovariectomy, all the rats were tested with an elevated T-maze (ETM), the well established model for measuring anxiety in rodents.

Results and Discussion

Four weeks after ovariectomy, the ratio of uterine weight to body weight of the vehicle treated-ovx rats (Veh) was decreased significantly when compared to estrogen treated-ovx rats (E_2) but not differed from genistein (Gen) or Daidzein (Dai) treated-ovx rats. As for the behavioral tests, we found that rats treated with E_2 and daidzein had lowered levels of anxiety compared to the vehicle group as indicated by the decreased latency time of avoidance 2 in the ETM. For the Gen group, the avoidance 2 tended to be lower than Veh but was not different from Veh, E_2 or Dai groups.



From these findings, we can conclude that the phytoestrogen, daidzein (0.5 mg/kg) can reduce anxiety when tested with elevated T-maze in comparable to estrogen (10 μg/kg) when given to ovx rats for 4 weeks. Moreover, it should be noted that genistein (0.5 mg/kg), another phytoestrogen, although it was not different from vehicle, tended to reduce anxiety, suggesting that higher dose of genistein may be required. Therefore, it is likely that phytoestrogen, for instance daidzein may be an alternative to estrogen in treating patients with conditions that estrogen is contraindicated since it has no effect on reproductive organs.

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P30 Preliminary Study of the Difference of Genome-Wide Methylation Profiles Between Germ Cells and Somatic Cells of Bulls

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Introduction and Objective

In this present study, we applied a PCR-based technique called Amplified methylation polymorphisms (AMPs) (1) to study the DNA mathylation profiles of germ cells (mature spermatozoa) and somatic cells (leukocytes and skin fibroblasts), in order to evaluate the degree of difference between the two cell lineages.

Materials and Methods

The samples came from 3 bulls. Mature spermatozoa was obtained from fresh ejaculations, leukocytes from whole blood and fibroblast cells from the culture of skin tissue were excised from the bull's ear. Genomic DNA was extracted from the samples and was digested with a methylation sensitive endonuclease enzyme (*Hpa*II). Both genomic- and digested-DNA samples were submitted for AMPs-PCR. Ten different oligo-nucleotide primers containing the HpaII recognition site (5'-CCGG-3') were used. The PCR amplicons were separated on to 4% polyacrylamide gels by electophoresis technique and were stained with silver nitrate. The results were evaluated on the presence-absence of markers between genomic- and digested-DNA templates. The differences of markers found between three cell types were calculated by Chi-square test.

Results

There are 3 types of AMPs markers; digestion resistant (R), digestion sensitive (S) and digestion dependent (D) (Fig. A,B and C). Samples from 3 bulls showed a similar but not identical pattern of DNA methylation in germ cells and somatic cells. The percentage of markers found in all samples is summarized in Table 1. Examples of methylation polymorphisms between samples are shown in Fig. 1 (D, E and F)

Table 1. Percentage of markers found in each cell type.

Marker	R	S	D
Sperm	92.3[a]	3.5	4.2[d]
Leukocyte	94.1[a]	2.6	3.3[d]
Fibroblast	90.1[b]	3.3	6.6[c]

*Different letters in the same column indicate significant difference (p<0.05)

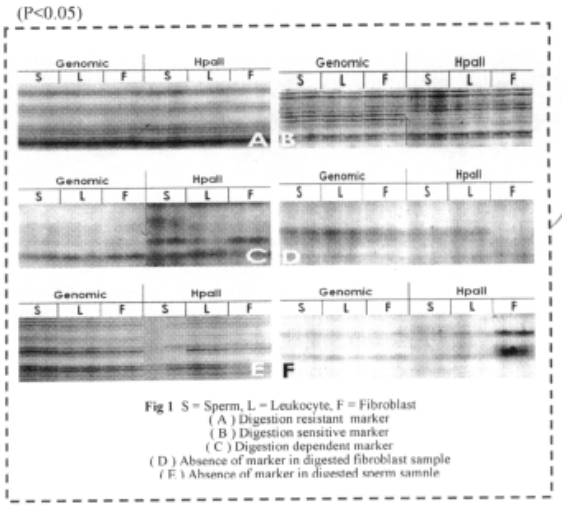

Fig 1 S = Sperm, L = Leukocyte, F = Fibroblast
(A) Digestion resistant marker
(B) Digestion sensitive marker
(C) Digestion dependent marker
(D) Absence of marker in digested fibroblast sample
(E) Absence of marker in digested sperm sample

Discussion and Conclusion

According to the high percentage of digestion resistant markers, the germ cells and somatic cells of bulls are highly methylated at the *Hpa*II sites with small variations both between the two cell lineages and within the somatic cell lineage. Markers found only in fibroblast cells may indicate the difference in genome structure between versatile and fully differentiated somatic cells.

Reference

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P31 Transient Translational Shutdown: An Element of Cellular Survival Strategies in Response to a Pore-Forming Toxin

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Introduction and Objective

S. aureus α-hemolysin is a cytotoxin that forms a small heptameric beta-barrel pore on plasma membrane of susceptible cells. Previous work of our group has demonstrated that the *c-fos* gene was rapidly upregulated 15 min after toxin treatment whereas *c-Fos* protein expression was observed after a lag of about 3 h. It was of interest to study how a delay of protein translation occurred after toxin treatment.

Materials and Methods

The skin keratinocyte cell line HaCat was treated with varying amounts of *S. aureus* α-toxin for the indicated time intervals.

In order to observe the delay of protein translation, the HaCat cells were exposed to 100 ng/ml α-toxin for 1 h. Subsequently, cells of 1 h were radiolabeled with [35S]Met for 30 min and harvested. The medium was changed and incubation was continued for another 3 h for cells for 4 h prior to being collected, separated by 12.5% SDS-PAGE, and visualized by autoradiography.

Lysates of cells treated with toxin were obtained *in situ* and subjected to SDS-PAGE and Western blot analysis. The expressions of phosphorylated eukaryotic initiation factor 2, alpha subunit (p-eIF2α) and total eIF2α proteins were observed.

Results and Discussion

Global translational repression was observed at 1 h after toxin treatment whereas at 4 h global protein synthesis resumed (Fig.1). The protein that might play an important role in repression of protein synthesis was explored. Phosphorylated eukaryotic initiation factor 2, alpha subunit, p-eIF2α, plays an important role in the shutoff of protein synthesis (1). eIF2α was found to be phosphorylated upon treatment with α-toxin to 30 min (Fig.2). Hence, we hypothesized that p-eIF2α expression led to the transient translational shutdown of HaCat cells treated with *S. aureus* α-toxin.

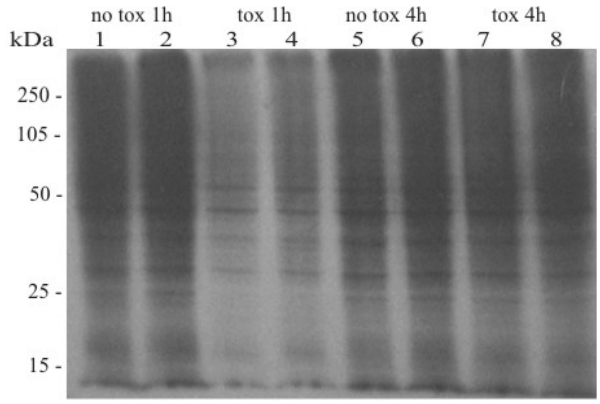

Fig.1. Transient translational shutdown in response to α-toxin treatment

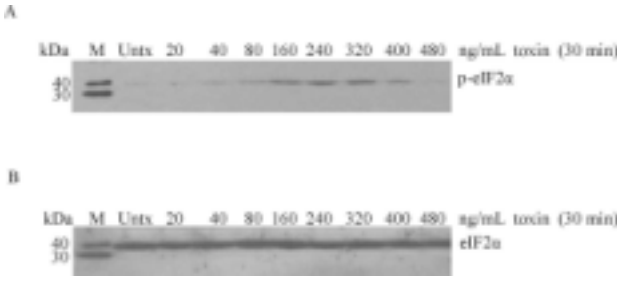

Fig. 2. α-Toxin rapidly induced eIF2α phosphorylation

Reference

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P32 The Development of Intra- and Inter-Species Cloned Embryos Derived from Rabbit Oocytes: The Effect of Donor Cell sources

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[1]Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, [2]Reproductive Medicine Unit, Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Introduction and Objective

Generating cloned embryos using rabbit oocytes as the recipient cytoplasm is useful in studying the interaction between a foreign donor nucleus and the recipient cytoplasm.

The aims of the study were to 1) investigate the development of inter-species cloned embryos derived from rabbit oocytes, using cows (no.1 and 2), swamp buffalo, pig and elephant ear fibroblasts as donor cells and 2) observe the developmental capacity of cloned rabbit embryo derived from adult and foetal fibroblasts.

Meterials and Methods

Mature oocytes were collected from superovulated rabbit does, by oviductal flushing. A single fibroblast (passage 3-5), in the starvation stage (1-3 days prior to nuclear transfer) was transferred into an enucleated oocytes. Reconstructed oocytes were later fused by electrical stimulation using 3 DC pulses, 3.2 kV.cm-1, for 20 μs.

Fused couplets were activated by inducing the same electrical stimulation, with subsequently incubation in activation medium, comprised of 5 μg.ml-1cyclo-heximide, 2 mM.ml- 6-DMAP and 10% foetal bovine serum (FBS), in synthetic oviduct fluid (SOF), for 1 h. They were then cultured in SOF supplemented with 10% FBS for 5 days.

Results and Discussion

The fusion rates of cow no.1 (62%; 82/133), cow no.2 (54%; 41/76), buffalo (63%; 53/84), pig (69%; 60/87), elephant (59%, 44/74), adult rabbit (55%; 41/74) and foetal rabbit couplets (70%; 56/80), did not significantly differ. The cleavage rates of cows no.1 and 2, buffalo, pig and elephant embryos were 33%, 83%, 57%, 67% and 100%, respectively.

The rabbit fibroblasts derived from adult gave a significantly lower cleavage rate than those derived from foetus (54% vs. 95%) ($P<0.05$). The developmental rates at morula and blastocyst of cloned embryos reconstructed from foetal rabbit fibroblasts were significantly greater than those reconstructed from adult rabbit, buffalo, pig and elephant (morula; 39%, 12%, 4%, 7% and 14%, blastocyst; 23%, 7%, 0%, 3% and 9%, respectively) ($p<0.05$).

While cow no.2 donor cells provided a significant embryo development success rate in term of blastocyst formation, cow no.1 cloned embryos could not develop beyond the cleavage stage (morula; 10% vs. 0% and blastocyst; 7% vs. 0%).

In conclusion, cow, swamp buffalo, pig and elephant fibroblasts can be produced by using enucleated rabbit oocytes as recipient cytoplasm. The developmental capacity of cloned embryos is affected by the sources, the individual ability of the donor cell used and varies according to the species.

This study was supported by Ratchadaphiseksomphot Endowment Fund, Reproductive Biotechnology Research Unit, Chulalongkorn University and Royal Golden Jubilee, PhD program of Thailand Research Fund. This work was presented at the Annual conference of the International Embryo Transfer Society, Kyoto, Japan, 6-10 January 2007.

P33 Inter-generic Marbled Cat and Flat-Headed Cat Cloned Blastocysts, Generated from Domestic Cat and Rabbit Oocytes

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Sumet Kamolnorranath[3] Kaywalee Chatdarong[2] Mongkol Techakumphu[2]

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Introduction and Objective

Marbled cats (*Pardofelis marmorata;* MC) and flat-headed cats (*Prionailurus planiceps;* FC), the endangered, small wild cats of south-east Asia, are currently at risk of becoming extinct in Thailand. Producing embryos by inter-generic somatic cell nuclear transfer (SCNT), is a way of preserving their genetics, as well as to understand their embryo chronology.

The aim of the study was to investigate the development of MC and FC embryos produced from domestic cat (DC) and rabbit (RB) oocytes by SCNT.

Materials and Methods

The MC-, FC-, DC-DC and FC-RB (donor fibroblast cell - recipient oocyte) couplets were prepared as previously described (1), but MC-, FC- and DC-DC couplets were fused by inducing 3 direct current pulses of 2.4 kV/cm, for 50 μs, and subsequently activated in synthetic oviduct fluid (SOF) medium, supplemented with 10% fetal bovine serum (FBS), 10 μg/ml cycloheximide and 5 μg/ml cytochalasin B, for 4 h.

Activated couplets were cultured in SOF medium supplemented with 5% FBS, at 38.5°C, under 5% CO_2 in air, for 5-7 days.

Intra-species cloned DC served as control. Differences in the percentages of fusion and embryo development to a particular stage between species were determined by chi-square analysis.

Results and Discussion

The fusion efficiency of DC-DC couplets (38%; 36/94) was significantly lower than those of MC-DC (74%; 60/81), FC-DC (79%; 60/76) and FC-RB (78%; 47/60) couplets ($p<0.05$).

Both cat and rabbit oocytes supported the remodeling of the FC nucleus. No differences in the cleavage rates of MC-DC, FC-DC, FC-RB and DC-DC fused couplets were found (93%, 97%, 87% and 89% respectively). The FC-DC couplets yielded a greater percentage of morula (53%) than those of MC-DC (23%), FC-RB (25.5%) and DC-DC (11%) couplets ($P<0.05$). However, the number of couplets that achieved blastocyst expansion did not significantly differ (MC-DC; 5%, FC-DC; 8.3%, FC-RB; 8.5% and DC-DC; 8.5%).

These results demonstrate that MC and FC embryos are successfully produced with reasonable blastocyst formation rates by inter-generic SCNT.

Reference

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This study was supported by the Zoological Park Organization under the Royal Patronage of H.M. the King, Reproductive Biotechnology Research Unit, Chulalongkorn University and Royal Golden Jubilee, PhD program of Thailand Research Fund. This work was presented at the Annual conference of the International Embryo Transfer Society, Kyoto, Japan, 6-10 January 2007.

P34 Maintaining the Meiotic Arrest of Cat Oocytes by a Specific Cyclin-Dependent Kinase Inhibitor

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Introduction and Objective

In vitro maturated cat oocytes demonstrate poor embryo development compared with those derived from in vivo. Inadequacy in cytoplasmic maturation due primarily to culture condition is one of many factors contributing to development incompetence. Roscovitine (ROS), a specific cyclin-dependent kinase inhibitor, has recently been shown in many species to maintain meiotic arrest without compromising the developmental competence of the oocytes. This study aimed to investigate the effect of ROS on the meiotic arrest of cat oocytes.

Materials and Method

Cumulus oocyte complexes (COCs) were recovered by slicing technique, and classified as good and poor quality COCs, in order to compare the effect of ROS on meiotic arrest. Groups of 20-30 COCs were cultured at 38.5°C, 5%CO2 in maturation medium containing with different concentration of (12.5, 25, 50, 100 and 200 μM). The COCs treated in 0 μM ROS served as controls. To assess probable toxicity of DMSO (solvent of ROS), the maximal concentration of DMSO used (2%(v/v) DMSO) was additionally examined. Overall, a total of 1,083 COCs were used in this experiment.

After 24h of culture, the COCs were denuded, fixed, stained with DNA labeling (4',6-Diamidino-2-phenyindole, DAPI), and the stage of nuclear maturation visualized using a fluorescent microscope.

Data was expressed as a mean ± SE. Differences among the experimental groups were assessed using a one-way ANOVA test and DUNCAN analysis. $p<0.05$ was considered statistical significance.

Result

In general, good quality COCs resumed and reached the metaphase II (MII) stage at greater rates than poor quality COCs (74.1 ± 6.0 vs. 32.8 ± 5.9). The 2% DMSO employed did not significantly affect the success of *in vitro* maturation rates (% MII: 80.3 ± 4.6 and 47.1 ± 6.0 for good and poor quality COCs, respectively). The COCs were cultured for 24 h in an absence of ROS (0 μM), they resumed and reached MII stage spontaneously (% GV: 24.1 ± 5.9 vs. 39.3 ± 6.3 for good and poor quality COCs, resp.), while markly ROS arrested the oocytes at an immature stage (germinal vesicle stage, GV) during 24 h of culture. This however, was the dose-dependent phenomenon. For good quality COCs, a large number of oocytes cultured in the presence of ROS (50, 100 and 200 μM) arrested at the GV stage (% GV: 85.7 ± 4.4, 87.2 ± 3.8 and 75.9 ± 4.7, resp.), while ROS at 12.5 and 25 μM yielded less efficiency ($p<0.05$). In addition, all chosen doses of ROS were capable of arresting the poor quality COCs at the GV stage (%GV ~ 70-90%).

Discussion

The results demonstrated convincingly that ROS arrested cat oocytes at the GV stage in a concentration-dependent manner. Although the 50, 100 and 200 μM ROS yielded similar GV rates, 100 μM ROS was the most efficient concentration due to the low % degenerate oocytes after culture, compared with those found in 50 and 200 μM. This was not the effect of DMSO per se since it did not affect the maturation rates. However, it is important to examine the developmental competence of these GV arrested oocytes after ROS treatment, in order to know the capability of the oocytes to reach MII stage, fertilize and develop up to embryo.

P35 ***In vitro*** **Activity of the Chicken Egg Antibody (IGY) Against *Lawsonia Intracellularis***

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Introduction and Objective

Lawsonia intracellularis (LI) is an obligate intracellular bacterium and the causative agent of proliferative enteropathy (PE), or ileitis, in pigs, as well as a variety of other species. The purpose of this study was to determine if LI-specific antibodies produced in chicken eggs reduce infection of cultured cells after challenge with LI.

Materials and Methods

LI, strain PHE/MN1-00, was cultured and served as the source of the antigen. Ten laying hens were inoculated with a 50:50 mixture of Freund's complete adjuvant and 108 formalinized LI antigen/ml biweekly for eight weeks. Another 10 laying hens were inoculated with the carrier alone. Eggs were collected daily post-inoculation and stored in -20°C until used. The eggs and hen sera were titered against a known concentration of LI antigen using a modified serology test. The anti-LI IgY was isolated from the eggs with the EGGstract IgY Purification System (Promega) and confirmed by SDS-PAGE electrophoresis and western immunoblotting analysis. To determine the immunological specificity of the anti-LI IgY, whole cell LI and LI flagellin protein (FliC) were electrophoresed in SDS-PAGE and transferred to PVDF membranes. The membranes reacted with anti-LI IgY, polyclonal rabbit antibody against whole cell LI, or swine-exposed LI sera (1:1000 dilution). Horseradish peroxidase labeled secondary antibody was added to elicit color development. To test the effect of anti-LI IgY on cultured cells infected with LI, McCoy cells were grown on glass slides in 6 well plates for one day. The cells were then infected with $2.5x10^2$ LI/ well in the presence or absence of anti-LI IgY (35 μg/ml).

Results

Following immunization, a serology of the hens revealed high antibody titers against LI up to 1:1,920 and even higher egg titers, between 1:1000 and 1:5000. The anti-LI IgY was isolated from eggs with the EGGstract IgY Purification System and confirmed to be LI-specific by SDS-PAGE and Western immunoblotting analysis. The purified protein contained two major components with molecular weights of about 25 kDa (IgY light chain) and 64 kDa (IgY heavy chain). Anti-LI IgY was shown to recognize whole cell LI and LI flagellin proteins (FliC). Similarly, the polyclonal rabbit antibody against whole cell LI and swine exposed LI sera recognized whole cell LI and LI flagellin proteins. Anti-LI IgY reduced the incidence of heavy LI infection in cultured cells after challenge with LI. Numerous LI accumulated in the cytoplasm of cultured cells were observed after infection with LI. However, the number of LI-infected cultured cells dramatically decreased in the presence of anti-LI IgY.

Discussion and Conclusion

Immunization of laying hens with whole cell LI caused specific antibodies to be present in their eggs. The production of LI-specific antibodies was confirmed by the fact that the anti-LI IgY recognized whole cell LI and LI flagellin. In this study, LI-specific antibodies produced in chicken eggs were able to reduce the incidence of infection of cultured cells after challenge with LI. Other reports noted that both a hamster model and a pig challenge trial demonstrated that feeding LI-specific egg antibodies can successfully reduce the production loss encountered as a result of LI infection.

Quality Control of the Formulated Clove Oil solution Aquanes®

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Introduction and Objectives

Anesthetics are used in aquaculture during transportation to minimize stress and reduce metabolism. They are also used to immobilize fish during fishery management; manual spawning, weighing, measuring, marking or veterinary practice. Many compounds have been evaluated to anesthetize fish effectively and some are being distributed commercially. Effects of clove oil to produce sedation and anesthesia have been reported in some fish spp. and abalone (1,2). The constituents found in clove oil are phenolic compounds, mainly eugenol. Because clove oil is not soluble and poorly distributed in water, the use of inadequately formulated clove oil solution results in different effectiveness. Using ethanol to dissolve clove oil may irritate and cause skin burn to fish. We have formulated AQUANES®, the ready-to-use clove oil solution for anesthesia of aquatic animals. The product is water-soluble and its safety and efficacy has been experimentally evaluated. As a corollary, in this study, the formulated AQUANES® was analyzed for the eugenol content as part of product quality control.

Materials and Methods

The formulated clove oil solution AQUANES®, equivalent to 5% eugenol was prepared from standardized clove oil raw material and a number of pharmaceutical excipients i.e. polyethylene glycol 400 (PEG), propylene glycol (PG), ethanol, methylparaben (MP), propylparaben (PP), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BTA), ethylenediaminetetraacetic acid (EDTA) and FD&C green. The formulated solution was subjected to quality control by determination of eugenol using gas chromatography (GC) method[3]. Standard eugenol and sample solutions in methanol were prepared to obtain eugenol concentration at 1.2 mg/ml, spiked with vanillin 2.0 mg/ml as an internal standard. GC analysis was performed on a PerkinElmer (Autosystem XL) equipped with a flame-ionization detector and a Stabiwax® capillary column (Crossbond(r) Carbowax® - PEG, 0.25 mm x 30 m I.D., 0.25 μm df). Injector and detector temperature were set at 250°C and the flow rate of helium gas was 1.5 ml/min. A 1 μL of each solution was manually injected onto the column under the oven temperature program described in Table 1.

Table 1. Gas chromatography oven temperature program for the analysis of eugenol.

Rate (°C/min)	Temperature (°C)	Hold (minutes)
0	160	1.50
20	200	0.00
40	220	3.00

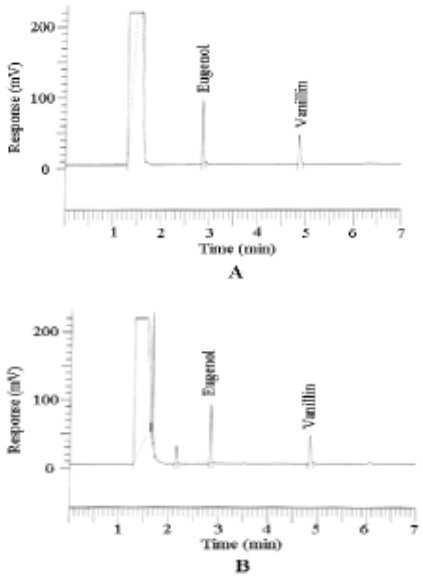

Figure 1. Typical GC chromatograms of standard (A) and AQUANES® solutions (B).

Results and Discussion

The GC chromatograms of standard and AQUANES® solutions are shown in Figure 1.The chromatogram demonstrates good separation of eugenol from other components existed in AQUANES®. Eugenol and vanillin were eluted at 2.84 and 4.84 min, respectively. The content of eugenol in AQUANES® was found at 4.93%, comparable to 98.59% of the labeled amount.

Acknowledgement

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References

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