การผลิตซีลีเนียมยีสต์และผลของซีลีเนียมยีสต์เปรียบเทียบกับซีลีเนียมอนินทรีย์ในอาหารต่อคุณภาพตัวอสุจิของสุกรพ่อพันธุ์

นางสาวประกายดอย ดิษยบุตร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2555

ORGANIC SELENIUM YEAST PRODUCTION AND ITS EFFECTS ON BOAR SPERM QUALITY COMPARISONS WITH INORGANIC SELENIUM IN DIET

Prakaidoy Ditsayabut

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

(Asst. Prof. Dr. Apichat Boontawan)

Chairperson

(Asst. Prof. Dr. Chokchai Wanapu)

Member (Thesis Advisor)

(Dr. Pakanit Kupittayanant, DVM)

Member

(Prof. Dr. Sukit Limpijumnong)

Vice Rector for Academic Affairs

(Asst. Prof. Dr. Suwayd Ningsanond)

Dean of Institute of Agricultural Technology

ประกายดอย ดิษยบุตร : การผลิตซีลีเนียมยีสต์และผลของซีลีเนียมยีสต์เปรียบเทียบกับซีลีเนียมอนินทรีย์ในอาหารต่อคุณภาพตัวอสุจิของสุกรพ่อพันธุ์ (ORGANIC SELENIUM YEAST PRODUCTION AND ITS EFFECTS ON BOAR SPERM QUALITY COMPARISONS WITH INORGANIC SELENIUM IN DIET) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.โชคชัย วนภู, 117 หน้า.

การศึกษาครั้งนี้ได้แบ่งการทดลองออกเป็นสองการทดลองคือการผลิตซีลีเนียมยีสต์ (Se-yeast)และการตรวจสอบคุณภาพของน้ำเชื้อของสุกรพ่อพันธุ์เมื่อให้อาหาร ทางการค้าที่มีการเสริมซีลีเนียมยีสต์ ยีสต์และซีลีเนียมอนินทรีย์

การทดลองที่หนึ่งเป็นการผลิตซีลีเนียมยีสต์โดยการคัดเลือกสายพันธุ์ยีสต์จำนวน 14 สายพันธุ์ โดยทำการเลี้ยงในอาหารที่มีการเสริมด้วยโซเดียมซีลีไนด์ความเข้มข้น 10 มิลลิกรัมต่อลิตร พบว่า *Saccharomyces bayanus* สามารถตรึงซีลีเนียมไว้ภายในเซลล์ได้มากที่สุดคือ 6.63 ไมโครกรัมต่อมิลลิลิตร และมีปริมาณซีลีเนียมต่อน้ำหนักแห้งสูงสุดที่ 2.51 ไมโครกรัมต่อมิลลิกรัม และเมื่อเลี้ยงในถังหมักขนาด 5 ลิตรพบว่าการเลี้ยง *S. bayanus* เป็นเวลา 48 ชั่วโมง มีปริมาณซีลีเนียมต่อน้ำหนักแห้งสูงที่สุดที่ 6.43 ไมโครกรัมต่อมิลลิกรัม และสามารถตรึงซีลีเนียมไว้ภายในเซลล์ได้มากที่สุดคือ 6.91 ไมโครกรัมต่อมิลลิลิตร ในการทดลองที่สองเป็นการตรวจสอบคุณภาพของน้ำเชื้อของสุกรพ่อพันธุ์เมื่อให้อาหาร ทางการค้าที่มีการเสริมซีลีเนียมยีสต์ ยีสต์และซีลีเนียมอนินทรีย์ในระยะสั้นเพื่อตรวจสอบคุณภาพน้ำเชื้อของสุกรพ่อพันธุ์โดยอาหารสูตรที่ 1 เป็นกลุ่มควบคุม และ 2 ผสมยีสต์ 0.60 มิลลิกรัมต่อกิโลกรัมอาหาร สำหรับสูตรอาหารที่ 3 4 และ 5 ผสมซีลีเนียมอนินทรีย์ที่ระดับ 0.15 0.45 และ 0.60 มิลลิกรัมต่อกิโลกรัมอาหารตามลำดับ ส่วนสูตรอาหารที่ 6 7 และ 8 ผสมซีลีเนียมยีสต์ที่ระดับ 0.15 0.45 และ 0.60 มิลลิกรัมต่อกิโลกรัมอาหารตามลำดับ จากการเก็บรวบรวมข้อมูลและวิเคราะห์ลักษณะน้ำเชื้อเช่น ความผิดปกติของตัวอสุจิ การมีชีวิตของตัวอสุจิ การเลื่อนที่ได้ ปริมาณ ความเข้มข้นและจำนวนอสุจิทั้งหมด พบว่าการเสริมซีลีเนียมยีสต์และซีลีเนียมอนินทรีย์มีแนวโน้มของการเพิ่มขึ้นและรักษาระดับเปอร์เซ็นต์การเคลื่อนที่ของตัวอสุจิ ปริมาณน้ำเชื้อของสุกรพ่อพันธุ์เมื่อเทียบกับสุกรพ่อพันธุ์ที่กินอาหารปกติ มากไปกว่านั้นเปอร์เซ็นต์ความผิดปกติของตัวอสุจิของสุกรพ่อพันธุ์ยังลดลงอีกด้วย แต่การเสริมซีลีเนียมไม่มีผลต่อเปอร์เซ็นต์การมีชีวิตของตัวอสุจิ ความเข้มข้นของตัวอสุจิและจำนวนตัวอสุจิทั้งหมดของน้ำเชื้อในสุกรพ่อพันธุ์ ขณะเดียวกันเมื่อสิ้นสุดการทดลอง

พบว่าการเสริมซีลีเนียมยีสต์และซีลีเนียมอนินทรีย์ไม่มีผลต่อค่าโลหิตวิทยาและค่าทางชีวเคมีในเลือดของสุกรพ่อพันธุ์

สาขาวิชาเทคโนโลยีชีวภาพ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2555 ลายมือชื่ออาจารย์ที่ปรึกษา_____

PRAKAIDOY DITSAYABUT : ORGANIC SELENIUM YEAST PRODUCTION AND ITS EFFECTS ON BOAR SPERM QUALITY COMPARISONS WITH INORGANIC SELENIUM IN DIET, THESIS ADVISOR : ASST. PROF. CHOKCHAI WANAPU, Ph.D., 117 PP.

SELENIUM YEAST/INOGANIC SELENIUM/BOAR/SPERM/BLOOD

In this study, two experiments were conducted to produce selenium enriched yeast (Se-yeast) and to investigate sperm quality of boars fed with diets supplemented with Se-yeast, yeast, and Na_2SeO_3, and of those fed with commercial feed.

In the first experiment, 14 yeast strains from fermentation process for Se-yeast production were screened. The *Saccharomyces bayanus* showed the highest selenium accumulation at 6.36 µg/mL and 2.51 µg/mg dry cell weights within 48 h by optimal Na_2SeO_3 addition at 10 mg/L. The Se-yeast was accumulated gradually with increasing DCW (6.43 µg/mg DCW), and the highest selenium level was achieved at 6.91 µg/mL in a 5 L fermentor for 48 h at 20 mg/L of Na_2SeO_3.

The second experiment was performed to evaluate the short-term effect of Se-yeast, yeast and Na_2SeO_3 on boar's sperm quality. A total of 24 boars were randomly assigned to eight treatment groups. The boars of diet 1 and diet 2, set as controls, were fed with a commercial diet and a commercial feed supplemented with 0.60 mg yeast/kg of diet, respectively. Diets 3, 4 and 5 contained the following supplements, 0.15, 0.45 and 0.60 mg Na_2SeO_3/kg of diet, respectively. Diets 6, 7 and 8 contained 0.15, 0.45 and 0.60 mg Se-yeast/kg of diet, respectively. Data on semen characteristics including sperm abnormalities, sperm viabilities, sperm motilities, volume, concentration and total sperm were collected and analyzed.

The result showed that the supplementation of Se-yeast and Na_2SeO_3 in boar diets was able to increase and maintain sperm motility and volume, which were higher than those of commercial feed ($P>0.05$). Moreover, the sperm abnormalities of boar were decreased whereas sperm viabilities, sperm concentration and total number of sperms were not significantly different ($P>0.05$) in all treatments.

Finally, the supplementation of Se-yeast and Na_2SeO_3 in boar diets did not affect hematological and biochemical values in boar's blood.

School of Biotechnology Student's Signature_____

Academic Year 2012 Advisor's Signature_____

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Prakaidoy Ditsayabut

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LIST OF ABBREVIATION

ANOVA	=	Analysis of varience
BUN	=	blood urea nitrogen
cm	=	centimeter
CFU	=	colony-forming units
CFU mL-1	=	colony-forming units per milliliter
ºC	=	Degree Celsius
DM	=	dry matter
g	=	gram
g dL-1	=	gram per deciliter
h	=	hour
Hb	=	hemoglobin
IU	=	International Unit
L	=	liter
L min-1	=	liter per minute
µL	=	microliter
µm	=	micrometer
µg mL-1	=	microgram per mililiter
mg	=	miligram
mg dL-1	=	miligram per deciliter
mg mL-1	=	miligram per mililiter
mg kg-1	=	miligram per kilogram

LIST OF ABBREVIATION (Continued)

mg L^{-1}	=	miligram per liter
mL	=	mililiter
mL L^{-1}	=	mililiter per liter
M	=	mola
Min	=	minute
mm	=	millimeter
nm	=	nanometer
No.	=	number
ppm	=	part per million
PBS	=	phosphate buffer saline
RBC	=	red blood cells number
RNA	=	Ribonucleic acid
rpm	=	Round per minute
rps	=	rotations per second
S.E.M.	=	standard error of the mean
SGR	=	specific growth rate
U.S.A.	=	United States of America
W	=	Watt

CHAPTER I

INTRODUCTION

1.1 Significance of the study

At high concentration, selenium (Se) is toxic and affects on central nervous system (Diaz-Alarcon et al., 1994). However, at low concentration, Se is an essential element for animal and human diets (Kiffney and Knight, 1990). Se has structural and enzymatic roles as antioxidant and catalyst for production of active thyroid hormone; Se needs for the proper functioning of the immune system and appears to be a key nutrient in inhibiting HIV progression to AIDS (Rayman, 2000). An elevated Se intake may be associated with reduced cancer risk. Se showed cancer protective effect and inhibition of the tumor cell invasion (Yin et al., 2009; Zeng and Combs, 2008). It is generally believed that the ingestion of organic Se compounds is better and safer than that of inorganic Se.

Some microorganisms produce biomass with high protein content and meanwhile transform inorganic Se into organic form. Organic Se complexes containing amino acids are considered to be the most bio-available for human and animals (Zhou et al., 2009). Under appropriate conditions, yeasts are capable of accumulating large amounts of trace elements such as Se and incorporating them into organic compounds (Suhajda et al., 2000). This mineral is absorbed during the yeast growth process. The ability of *Saccharomyces cerevisiae* to transform inorganic Se into organic Se compounds is known and it is dependent on growth conditions. Yeast

is able to accumulate high concentrations of Se (3 mg/g) (Korhola et al., 1986) and transform inorganic Se mainly into selenomethionine (Se-met) (Ponce de León et al., 2002; Reyes et al., 2004). Continuous fermentations in optimum medium of sodium selenite (Na_2SeO_3) demonstrated higher biomass and Se content compared to the fermentation with sodium selenate (Na_2SeO_4) (Ali Demirci et al., 1999). Yeast is known for its high protein content; therefore, more Se can be incorporated by replacement of sulphur in proteins. Also, the industrial production of yeast is more manageable than the industrial production of Se enriched plants. The *Saccharomyces* clade is composed of seven species: *S. bayanus*, *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus* and *S. pastorianus*. Among these species, *S. cerevisiae* and *S. bayanus* are known for their role in alcoholic fermentation. *S. bayanus* has some specific properties: cryotolerance, production of smaller amounts of acetic acid and ethanol, but higher amounts of glycerol and succinic acid (Christine Le Jeune et al., 2007).

Se is a trace mineral required by boar that must be supplemented in the diet. It is an integral part of several enzyme systems in the body, including glutathione peroxidase (PGx). Peroxides are highly reactive molecules that form in the body as by-products of normal metabolism. If they are not removed or made less reactive, peroxides will damage cell membranes, resulting in reduced performance of cell.

Se deficiency symptoms continue to be reported in boar, including sudden death, Mulberry heart disease (MHD) in pigs, is characterized by lesions of acute haemorrhagic myocarditis and myocardial necrosis, poor growth, gastric ulcers, muscular dystrophy and abnormal sperm cells in boars. Se deficiency may be more common when Na_2SeO_3 serves as the major source of total dietary Se, because

Na_2SeO_3 is not stored in body tissues of pigs.

Na_2SeO_3 is easily absorbed by boar but is poorly retained in body tissues resulting in high levels of Se excreted in urine. Se-met is not as well absorbed from the digestive tract resulting in higher levels of Se excreted in feces. However, once absorbed Se-met is incorporated into body proteins in liver and muscle resulting in higher Se retention (www.akey.comwine).

Besides many other functions Se and its synergistic counterpart are crucial for the processes of reproduction for both males and females. A positive effect of Se was found in relation to sexual activity of boars their semen quality (motility, concentration, and sperm morphology) as well as to fertilization rates (Hansen and Deguchi, 1996; Marin-Guzman et al., 2000). As a source of Se the cited authors used inorganic compounds Na_2SeO_4 and Na_2SeO_3. Also in practicaly feeding of livestock animals including swine, inorganic compounds of this microelement are used.

1.2 Research objectives

The purposes of this study were as follows:

1. To produce organic Se- yeast using single dose addition of Na_2SeO_3.

2. To compare boar sperm quality of boar fed diet supplement with Se-yeast and inorganic Se.

1.3 Research hypothesis

The boar diets could be supplement with Se-yeast. These diets could be effect on reproductive system of boar.

1.4 Scope and limitation of the study

The optimum condition of Se will be study for organic Se production by yeast in 5 L fermentor. Organic Se and inorganic Se will be tested efficiency in boars reproductive.

1.5 Expected results

The high efficient Se-yeast obtains from yeast culture and shows high efficiency of Se-yeast on sperm quality after boar diet Se-yeast.

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CHAPTER II

LITERATURE REVIEWS

2.1 The general concept of selenium production

Selenium ("selene" in Greek meaning "moon") was discovered in 1817 by Jöns Jakob Berzelius, who found the element associated with tellurium (Te, atomic number 52), which was a byproduct of sulfuric acid production. Selenium (Se) is a chemical element with the atomic number 34 and atomic mass of 78.96 represented by the chemical symbol Se. It is a non metal and rarely occurs in its elemental state in nature. Isolated Se occurs in several different forms. The most stable of which is a dense purplish-gray semi-metal (semiconductor) form that is structurally a trigonal polymer chain. Se salts are toxic in large amounts, but trace amounts of the element are necessary for cellular function in most. Se is an essential micronutrient for animals but toxic in large dose. It is a component of the unusual amino acids selenocysteine and selenomethionine (Se-met).

Se is divided into two groups organic Se and inorganic Se (www.wikipedia. org). Organic Se is commonly found in plants and animals. It has been take up from the soil and corporate into some amino acids. This is the form which is digested and used efficiently. Inorganic Se especially sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) are actually byproducts of copper-mining. Organic Se, such as Se-yeast, is the product of the aerobic fermentation of *Saccharomyces cerevisiae* in Se-enriched medium. The medium is usually beet or cane molasses to which is added vitamins, nutritional salts and other growth factors to ensure maximal biomass and

measured amounts of Se salts as the Se source. Control of pH, temperature, Se feeding profile and aeration allows optimal growth of the yeast strain and maximum biomass production were studied by (Johnson, D.R., 2007).

2.2 Selenium in human health

There have been trials conducted on the effects of Se supplementation on the incidence of cancer in human subjects and all of them have shown positive effects of Se. The first intervention trial to prevent cancer with Se in human subjects was conducted in China, where there is a high incidence of primary liver cancer. Subjects were given 30–50 mg Se/d (3,658 subjects) for 8 years during1984 to 1991 (Yu et al., 1991 and 1997). This resulted in a drop of the primary liver cancer incidence to almost one-half. Upon withdrawal of Se from the treated group, the primary liver cancer incidence began to rise. The results indicated that a treatment containing Se (50 mg Se/d as Se-yeast plus vitamin E and β-carotene) produced a protective effect against esophageal and stomach cancer mortality among subjects in the general population (Taylor et al., 1995; Blot et al.,1995).

Table 2.1, shows daily intake levels of Se recommended by a number of national or international committees while Table 2.2, is a compilation of up-to-date Se intake data for a number of countries.

Table 2.1 Current recommended selenium intakes for adults (mg/d).

Country or region	RDA, RNI, PRI or NR	
	Males	**Females**
Australia, 1990	85	70
Belgium, 2000	70	70
DACH (Germany, Austria, Switzerland), 2000	30-70	30-70
France, 2001	60	50
Italy, 1996	55	55
Japan, 1999	55-60	45
New Zealand and Australia (proposed levels)	65	55
Nordic countries, 1996	50	40
USA and Canada, 2000	55	55

Source: Thomson, 2004

RAD, recommended dietary allowance; RNI, reference nutrient intake; PRI, population reference intake; NR, nutrient requirement.

Table 2.2 Diary selenium intake data for a number of countries.

Country	Se intake (mg/person/d)	Reference
Australia	57-87	Fardy et al. (1989)
Austria	48	Simma and Pfannhauser (1998)
Belgium	28-61	Simma and Pfannhauser (1998)
Czech Republic	10-25	Robberecht et al. (1994)
Canada	98-224	Kvícala et al. (1996)
China	7-4990	Gissel-Nielsen (1998)
Croatia	27	Combs (2001)
France	29-43	Lamand et al. (1994)
Germany	35	Alfthan and Neve (1996)
Japan	104-199	Miyazaki et al. (2001)
Netherlands	39-54	Van Dokkum (1995)
New Zealand	55-80	Vannoort et al. (2000)
Poland	30-40	Wasowicz et al. (2003)
Serbia	30	Djujic et al. (1995)
Slovakia	38	Kadrabova´et al. (1998)
Sweden	31	Becker (1989)
Switzerland	70	Kumpulainen (1993)
USA	106	Food and Nutrition Board (2000)

2.3 Selenium deficiency

In human beings, an association between low Se status and Keshan disease, a cardiomyopathy endemic to parts of China, was documented in 1979. Human Se deficiencies are rare. The primary groups of people who have developed Se efficiency have been those receiving total parenteral nutrition without Se for extended periods (Brown et al., 1986; Kien and Ganther 1983; Lockitch et al., 1990; Van Rij et al., 1979).

Although Se deficiency is rare, it may exacerbate damage from other disease causing factors. For example, coxsackie viruses have been implicated as possible cofactors in the etiology of the Se-responsive cardiomyopathy (the measurable deterioration of the function of the myocardium (the heart muscle) leading to heart failure) in Keshan disease (Beck et al., 1994).

2.4 Selenium toxicity

Residents had classic symptoms, such as changes in skin (red, swollen and listered), hair, nails (brittle, discolored and eventual loss), and nervous system unction (Yang et al., 1983). The most severe cases of intoxication involved changes in nervous system function and included peripheral anesthesia, convulsions, paralysis, and motor disturbance. Other symptoms of toxicity include anorexia, abdominal pain, diarrhea, fatigue, irritability, depression, emaciation, pulmonary edema, hemorrhage, liver and kidney necrosis, garlic/sour milk breath odor, neurologic deterioration, lindness, ataxia, respiratory distress and dental caries (Fan and Kizer, 1990; Elzlsouer et al., 1985).

2.5 Selenoproteins

Selenoproteins are proteins that contain the selenocysteine form of Se, now recognized as the 21st amino acid. Eleven selenoproteins have been identified: glutathione peroxidases (GPx), phospholipid hydroperoxide, thioredoxin reductase and selenophosphate synthetase. In addition, protein in the sperm mitochondrial capsule has been identified as a structural selenoprotein and is reported to contain 3 selenocysteine residues. Evidence suggests that this protein may be phospholipid, hydroperoxide, GPx rather than a structural selenoprotein (David et al., 1999).

2.5.1 Glutathione peroxidases

Se is probably incorporated into rate spermatocyte or early spermatids in the rat (Brown and Burk, 1972). Se has been found in high concentrations in testes and epididymides of boars, and is therefore likely important for the production and maturation of sperm (Lasota et al., 2004). Rotruck et al. (1973) demonstrated that Se is a component of GPx, an enzyme protecting cellular and subcellular membranes against peroxidation (Flohé et al., 1973). Nevertheless, the way GPx acts on sperm protect ion remains unclear.

While some authors demonstrated GPx activity in sperm of boars, others demonstrated that, in mammals, the enzyme loses activity once it is taken up in the sperm as a structural component (Ursini et al., 1999).Although each of the 4 enzymes is distinct, in general, GPx catalyze the reduction of peroxides that can damage cells and tissues. Se, therefore, as part of GPx, is considered one of the antioxidant nutrients and has interdependent roles with vitamin E, iron (ascatalase), and zinc and copper (as superoxide dismutase). These antioxidants help prevent the generation of free radicals and decrease the risk of oxidative damage to tissues. Free radicals have been

implicated in the etiology of many diseases as they can cause damage to DNA, proteins, carbohydrates and lipids throughout the body.

2.5.1.1 Cellular, glutathione peroxidase

Cellular GPx occurs in virtually all cells, which reduces hydrogen peroxide and free organic hydroperoxides to water and alcohols, respectively (Burk and Hill, 1993; Hoekstra, 1975). Cellular GPx may also represent a storehouse for Se that can be used for other purposes (Burk and Gregory, 1982; Yang et al., 1990).

2.5.1.2 Plasma or extracellular glutathione peroxidase

Plasma or extracellular GPx is also found in human milk and has been found to be synthesized primarily in the proximal tubular cells of the kidney. Plasma GPx activity is diminished during Se deficiency and plateaus when adequate intakes are reached (Bhattacharya et al., 1988; Avissar et al., 1994; Whanger et al., 1998).

2.5.1.3 Phospholipid hydroperoxide glutathione peroxidase

Phospholipid hydroperoxide GPx functions to reduce fatty acid hydroperoxides that are esterified to phospholipids. It has also been shown to reduce the hydroperoxides of cholesterol and cholesterol ester in membranes. It has been suggested that phospholipid hydroperoxide GPx may be the selenoprotein present in the sperm mitochondrial capsule (Roveri et al., 1992;1994).

2.5.1.4 Gastrointestinal glutathione peroxidase

Gastrointestinal glutathione peroxidase is the major GPx activity in the gastrointestinal tract and plays an important role in protecting mammals from the toxicity of ingested lipid hydroperoxides (Chu et al., 1993).

2.5.2 Thioredoxin reductase

Thioredoxin reductase was illustrated selenium's role in another major antioxidant system. Low levels of Se, leading to decrease thioredoxin reductase activity, may be related to a cellular ability to undergo apoptosis, which increases the risk of cancer (Oblong et al., 1993; Gallegos et al., 1997).

2.5.3 Selenoprotein P

Selenoprotein P is a glycoprotein that carries up to 50% of the Se in plasma. The function of selenoprotein P is unknown, it has been hypothesized to have a role in transport and oxidant defense. Selenoprotein P has been demonstrated to deliver Se preferentially to the testes in Se deficient rats (Wilson and Tappel, 1993).

2.5.4 Selenoprotein W

Selenoprotein W is essential for the maintenance of normal liver function, the expression of Selenoprotein W liver depends on the level of selenium supplied with the diet. Selenoprotein W is involved in muscle growth and differentiation by protecting the developing myoblasts from oxidative stress (Loflin et al., 2006).

2.5.5 Sperm Mitochondrial Capsule (Selenoprotein)

Spermatozoa contain the highest concentration of Se of any tissue in the mammalian body. A Se-containing polypeptide found in rat spermatozoon within a keratinous fraction mitochondrial capsule of the sperm (Calvin, 1978). This protein is localized in the midpiece portion of the spermatozoon, is associated with a specific keratin within a fibrous raction of the sperm tail (Brown snd Burk, 1973; McConnell and Burton, 1981), and is necessary for the flagella integrity of the sperm. The protein has been reported to contain 3 selenocysteine residues (Karimpour et al., 1992). Se

deficiency negatively affects the synthesis of the sperm mitochondrial capsule and may result in sterility because of reduced sperm motility and impaired spermatogenesis. The Se content of human gonads increases during sexual maturation in men, whereas serum Se concentrations have been shown to decrease. An increased demand for Se with the onset of spermatogenesis, along with a possible inadequate dietary intake of Se during puberty, could explain the decrease in the circulating Se level. Supplementation studies have indicated that infertile men supplemented with Se and vitamin E showed improvement of sperm motility, vitality, and morphology. Subjects supplemented with sodium selenite showed increased Se concentrations in the seminalfluid; only those supplemented with Se yeast also exhibited an increase in seminal glutathione peroxidase activity. Controversy currently exists about whether the sperm selenoprotein is unique or simply phospholipid hydroperoxide glutathione peroxidase.

2.6 Inhibition of genetic damage by Se

Se deficiency has been implicated as playing a role in the development of many diseases, including cancer, cardiovascular and immune disorders (El-Bayoumy, 2001). On the other hand, the intake of an excess of Se may result in oxidative damage leading to genomic instability (Spallholz, 1994). However, that levels of Se inhibition of genetic damage and cancer in both rodents and in humans (El-Bayoumy, 1999).

Low Se intakes have been linked to an increased risk of cancer, and many Se compounds given at levels that exceed nutrition needs have been found to inhibit tumor growth in animal models. A 10-year clinical trial suggested a lower risk of cancer associated with the use of a 200 µg Se supplement as Se-yeast. Although Se

treatment did not protect against development of basal or squamous cell carcinomas of the skin, supplemental Se was associated with reductions in lung, colorectal, and prostate cancer incidences (Davis et al., 1996). Evidence indicates that are multiple mechanisms by which Se can inhibit carcinogenesis. However, the dose response relationship between Se and tumor growth is not linear. The antitumorigenic effects of Se in animal models have been observed at levels greater than those required to maintain selenoproteins. It appears that the primary mechanisms by which Se inhibits carcinogenesis involve alterations in carcinogen metabolism or production of toxic Se metabolites distinct from the known selenoproteins. The role of Se in thioredoxin reductase is one of many ways which Se may act as a chemopreventive agent.

2.7 The protective role of Se in cancer prevention

Clinical intervention studies

The cancer prevention trials have shown that giving a combination of Se, β-carotene and α-tocopherol resulted in significantly fewer cases and a lower mortality from stomach cancer than were observed in the placebo groups (Blot et al., 1993). When Se was given in combination with another 25 vitamins and minerals, it had no effect on the development of esophageal cancer. In a study conducted in India, Se was given in combination with vitamins A, C and E, as well as zinc. Here, the results clearly showed a protective effect of this cocktail against the development of oral lesions in subjects who practice reverse smoking (Parsad et al., 1995). One of the most exciting clinical trials in the United States of America supported a protective effect of Se-enriched yeast against cancer of the prostate, colon, and lung (Bonelli, 1998; Clark et al., 1996; 1998; Li et al., 1993).

2.8 The protective role of Se on oxidative damage

Protection against oxidative damage by Se has been proposed because it is a component of GPx and other Se-containing enzymes. However, evidence for the role of Se-containing enzymes in cancer prevention is not clearly defined. It is of significance that Se has been shown to inhibit oxidative damage to lipids, proteins, and DNA in rodents (Rosa et al., 1998; El-Bayoumy et al., 2000) similar studies in humans (Prasad et al., 1995). Whether reduction of lung, colon and prostate cancers in the clinical trial that employed Se-yeast is due in part to a reduction in levels of various kinds of oxidative damage (Clark et al., 1998).

The data clearly showed that the dose and formulation of Se compounds are critical determinants with regard to cellular responses. Inorganic Se compounds appear to cause distinctly different cellular effects from those elicited by organic forms of Se in vitro and in vivo in preclinical and clinical investigations (Biswas et al., 1999). Clearly, Se compounds are capable of inhibiting, carcinogen-induced covalent DNA adduct formation and DNA oxidative damage, DNA methylation, micronuclei induction, chromosomal aberrations and cancer (An et al., 1998; Sinha et al., 1999; Biswas et al., 1999;).

2.9 Toxicity of Se in animals

Acute toxicity studies with Se-yeast have been carried out on rats (Vinson and Bose, 1987). The LD_{50} for Se-yeast was 37.3 mg/kg compared with 12.7 mg/kg for Na_2SeO_3 demonstrating that Se-yeast is considerably less acutely toxic about three times than sodium selenite.

A study comparing the toxicity of $Na_2SeO_3$and Se-yeast diets in rats also showed that Se-yeast was much less toxic than Na_2SeO_3 and than severe hepatotoxicity, cardiotoxicity and splenomegaly were observed in rats fed Na_2SeO_3 at levels of 16 mg Se/g diet over an 8 week period whereas animals fed high Se-yeast at the same level showed no such symptoms. Although the livers of animals fed Se-yeast showed up to 50% greater deposition of Se. There was no corresponding toxicity as evidenced by histological examination (Spallholz and Raftery, 1987).

Se has been considered an essential element since Schwarz and Foltz demonstrated in 1957 that it was the effective component of "factor-3" (Schwarz, 1951) in preventing liver degeneration in rats. Se showed to prevent exudative in chicks and nutritional muscular dystrophy in calves and lambs. The studies with Se involving other species (Table 2.3) and other deficiency syndromes against which Se and vitamin E may be effective have been reviewed (Ammerman and Miller, 1975).

Table 2.3 Dietary requirements and toxic levels of Se for domestic animals.

Species	Se (ppm)	
	Requirement	**Toxicity**
Dairy cattle	0.1	5
Sheep	0.1	3-20
Swine	0.1	5-10
Chickens (0 to 8 wk)	0.1	10

Source : Ammerman and Miller, 1975

2.10 Anatomy and Physiology of Boar

The male reproductive system consists of two testicles, each of which is held almost vertically with the tail of the epididymis at the top (Fig. 2.1). The epididymis is the area within which all the mature sperm is stored and held until ejaculation. From each testicle a tube, the urethra, carries the sperm into the abdomen via the inguinal canal. From there it enters the neck of the bladder and continues in the groin down the penis to the exterior as the urethra.

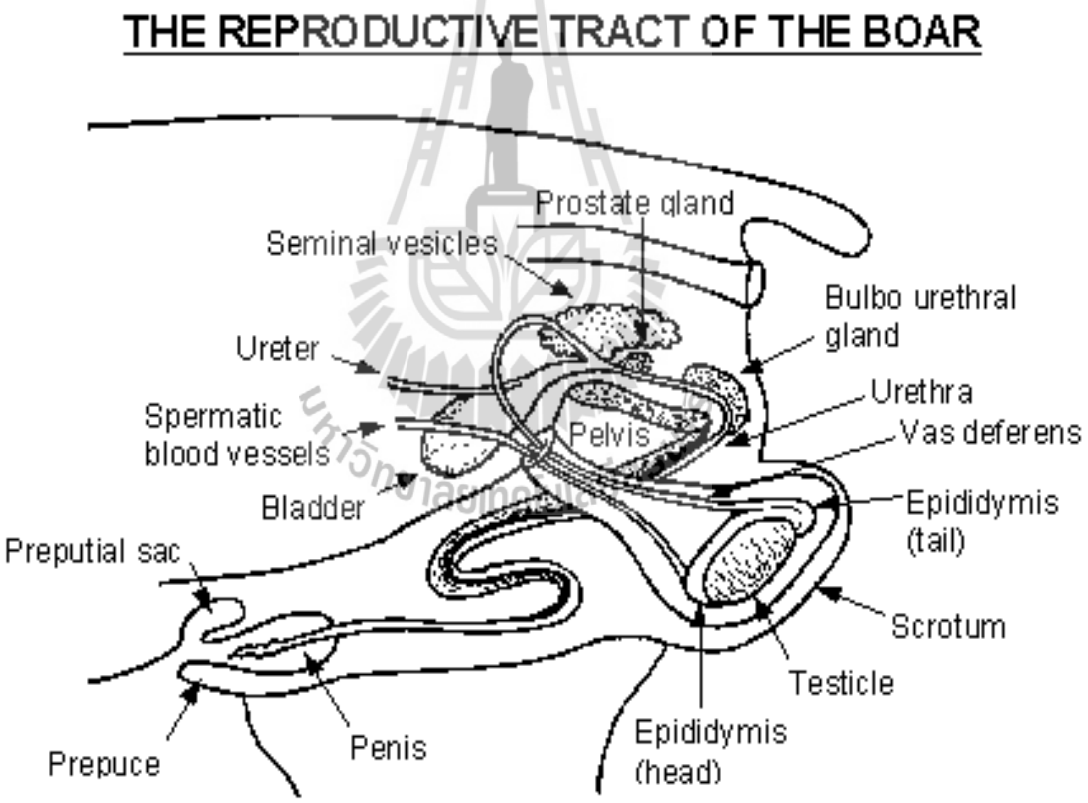

Figure 2.1 The reproductive system of boar

Source: Thepigsite, www. 2012

Thus from the neck of the bladder to the tip of the penis the urethra can carry either sperm or urine. There are three glands called the seminal vesicles, the prostrate and the bulbo urethral glands. The seminal vesicles produce the bulk of the ejaculate (300 mL) and fructose to nourish the sperm. The prostate gland provides other nutrients and the bulbo urethral gland the jelly that you often see at the end of mating. During service the sperm in the epididymis are pulsated down the urethra to be joined by the seminal fluids. This is a continuous process during the period of mating.

The penis, which is long and rigid, has a sigmoid or S-shape in its top half and an anti clockwise spiral at the end. It is 300-500 mm long. Sperm is produced and matured under the influence of luteinizing hormones (LH) and follicle stimulating hormones (FSH) and the whole process is controlled by the pituitary gland which is at the base of the brain.

Spermatogenesis occurs in the hundreds of seminiferous tubules of the testes, and is dependent on the actions of testosterone produced from cells which lie among these tubules (Leydig cells) and of the gonadotrophic hormones from the pituitary gland. It begins at puberty when the germ cells (spermatogonia), which have been in the testes since fetal life, start dividing by mitosis to produce a small clone of daughter cells with the normal 23 pairs of chromosomes (diploid cells).

One of these pairs constitutes the sex chromosomes: in males an X chromosome and a smaller Y chromosome, which carries the male-determining gene. The majority of these cells (now termed primary spermatocytes) push their way through the junctions between the large protective and nourishing cells (Sertoli cells) which lie between them and the lumen of the tubule. In their new environment, created by secretions of the adjacent Sertoli cells (Fig 2.2), they undergo divisions

which halve their number of chromosomes. In the first meiotic division, the pairs of chromosomes come together and strands of DNA are swapped between them (crossing over), thus changing the genetic code carried by each chromosome. Eventually the pairs separate and two haploid cells, each containing a single set of 23 chromosomes, are formed. Thus one of these 'secondary spermaocytes' contains an X chromosome and the other a Y chromosome.

Almost immediately after this first meiotic division a second meiotic division takes place. This involves the separation of the two halves of each single chromosome. These haploid cells now called spermatids thus contain 23 single half chromosomes. By this stage the important genetic events have taken place, but these spermatids are still simple round cells and must now undergo extensive remodelling (spermiogenesis) before they are capable of performing their function.

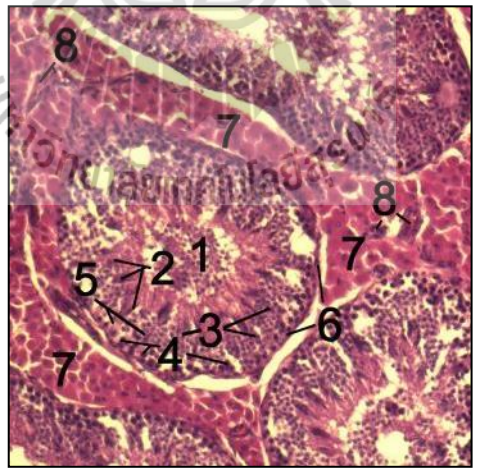

Figure 2.2 Histological section through testicular parenchyma of a boar,
1) Lumen of Tubulus seminiferus contortus, 2) spermatids,
3) spermatocytes, 4) spermatogonia, 5) Sertoli cell,
6) Myofibroblasts, 7) Leydig cells, 8) capillaries
Source: Wikipedia, www. 2012

Spermiogenesis is the final stage of spermatogenesis, which sees the maturation of spermatids into mature, motile spermatozoa. The spermatid is more or less circular cell containing a nucleus, Golgi apparatus, centriole and mitochondria. All these components take part in forming the spermatozoon. The process of spermiogenesis is traditionally divided into four stages: the Golgi phase, the cap phase, formation of tail and maturation stage (Fig 2.3).

2.10.1 Golgi phase

The spermatids, which up until now have been mostly radially symmetrical, begin to develop polarity. The head forms at one end, and the Golgi apparatus creates enzymes that will become the acrosome (Fig. 2.3). At the other end, it develops a thickened mid-piece, where the mitochondria gather and the distal centriole begins to form an axoneme. Spermatid DNA also undergoes packaging, becoming highly condensed. The DNA is packaged first, with specific nuclear basic proteins, which are subsequently replaced with protamines during spermatid elongation. The resultant tightly packed chromatin is transcriptionally inactive.

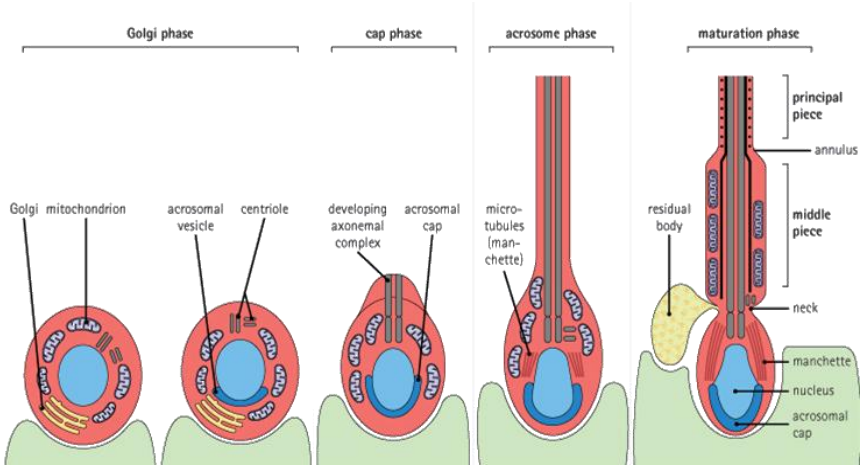

Figure 2.3 The process of spermiogenesis

Source: steinbachs, www. 2012

2.10.2 Cap phase

The Golgi apparatus surrounds the condensed nucleus, becoming the acrosomal cap.

2.10.3 Formation of tail phase

One of the centrioles of the cell elongates to become the tail of the sperm. A temporary structure called the "manchette" assists in this elongation. During this phase, the developing spermatozoa orient themselves so that their tails point towards the center of the lumen, away from the epithelium.

2.10.4 Maturation phase

The excess cytoplasm, known as residual bodies, is phagocytosed by surrounding Sertoli cells in the testes. The mature spermatozoa are released from the protective Sertoli cells into the lumen of the seminiferous tubule and a process called spermiation then takes place, which removes the remaining unnecessary cytoplasm and organelles. The resulting spermatozoa are now mature but lack motility, rendering them sterile. The non-motile spermatozoa are transported to the epididymis in testicular fluid secreted by the sertoli cells with the aid of peristaltic contraction (Fig 2.4).

Whilst in the epididymis, they acquire motility. However, transport of the mature spermatozoa through the remainder of the male reproductive system is achieved via muscle contraction rather than the spermatozoon's motility. A glycoprotein coat over the acrosome prevents the sperm from fertilizing the egg prior to traveling through the male and female reproductive tracts. Capacitation of the sperm by the enzymes FPP (fertilization promoting peptide, produced by the male)

and heparin (in the female reproductive tract) remove this coat and allow sperm to bind to the egg.

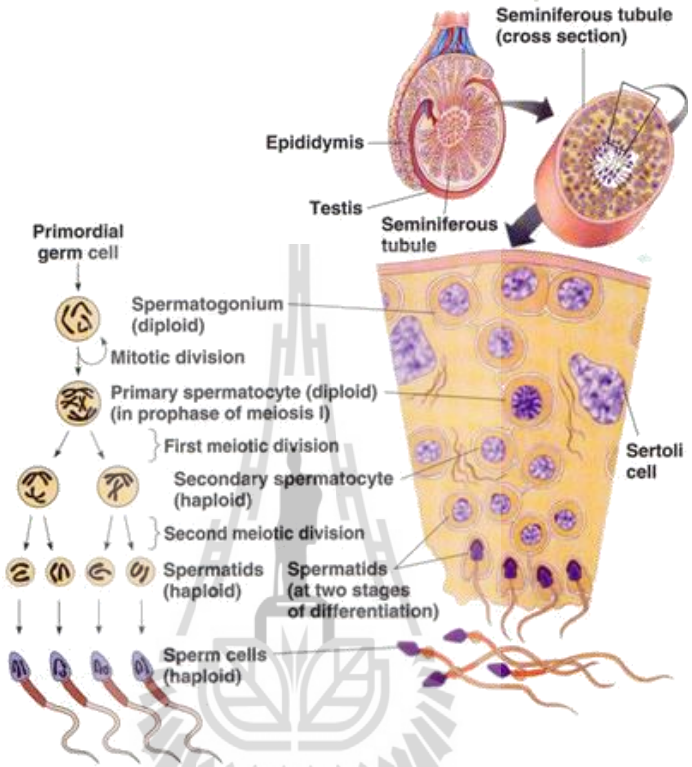

Figure 2.4 A mature sperm and sections of the testis and seminiferous tubules

Source: captain-nitrogen, www. 2012

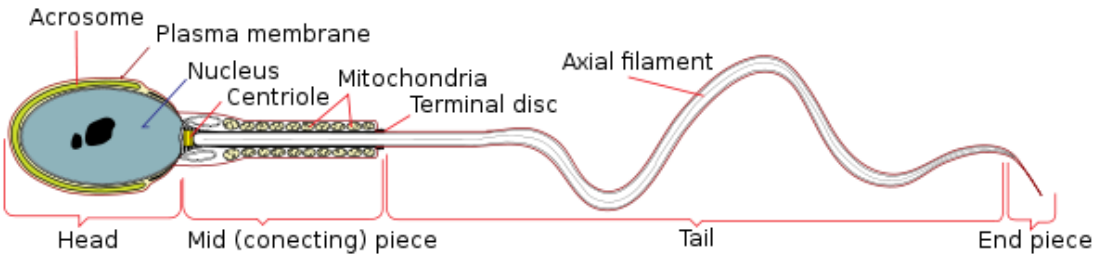

Figure 2.5 The mature sperm cell.

Source: Wikipedia, www. 2012

Table 2.4 Biochemical parameters in sperm and plasma fractions of semen.

Composition	Bull	Boar	Ram	Stallion
Ejaculate volume (mL)	5-8	0.8-1.2	150-200	60-100
Sperm concentration (million/mL)	800-2000	2000-3000	200-300	150-300
Sperm/ejaculate (billion)	5-15	1.6-3.6	30-60	5-15
Motile sperm (%)	40-75	60-80	50-80	40-75
Morphologically normal sperm (%)	65-95	80-95	70-90	60-90
pH	4-7.8	5.9-7.3	7.3-7.8	7.2-7.8
Fructose	460-600	250	9	2
Sorbitol	10-140	26-170	6-18	20-60
Citric acid	620-806	110-260	173	8-53
Inositol	25-46	7-14	380-630	20-47
Glyceryl phosphoryl choline (GPC)	100-500	1100-2100	110-240	40-100
Magnesium	8±0.3	6±0.8	5-14	9
Chloride	174-320	86	260-430	448
Ergothioneine	0	0	17	40-110
Sodium	225±13	178±11	587	257
Potassium	155±6	89±4	197	103
Calcium	40±2	6±2	6	26

Source: Hafez and Hafez (2000)

2.11 Environmental factors affecting on reproductive of boar

2.11.1 Acute stress

In terms of reproduction, these hormones can either stimulate or inhibit contractions and motility of the muscles of the reproductive tract. This, in turn, affects the movement of sperm and eggs for fertilization, the timing of embryos reaching the uterus and the number of embryos that actually survive to implantation.

2.11.2 Chronic stress

n boars, sperm maturation in terms of an increase in the percentage of sperm with cytoplasmic droplets, is one of the first signs of stress. Overall, the outcome of chronic stress or acute stress applied at critical stages of reproduction shows up as increased nonproductive days, reduced litter sizes, and higher replacement rates. It is often difficult to identify the cause for changes in these performance parameters, but investigation of the animal environment and its comfort level in that environment will usually prove beneficial.

2.11.3 Animal-animal interactions

Pigs being social animals, which in nature live in groups, require appropriate social cues during development for normal attainment of puberty and subsequent sexual behaviour. Gilts reared in groups with other gilts and exposed to boars as puberty approaches, reach puberty sooner, have stronger mating responses, are more interested in the boar and are more likely to stand for the boar than are those animals raised without these interactions (Hemsworth et al., 1982; Varley, 1994). The importance of the boar stimulus in the rearing environment should not be underestimated. Lack of this stimulus can lead to delayed age at conception, decreased farrowing rates and smaller litter sizes.

While the volatile chemical messengers, pheromones, from mature boars can be particularly stimulatory for young gilts, the greatest effect is when the boar is in direct, daily contact with the prepubertal gilts for a brief period of 15 to 30 minutes (Hemsworth et al., 1982). This may be interpreted as a stimulatory stress response. However, it can be counterproductive if initiated too early when the gilts cannot respond sexually or if continued for too long.

Boar sperm production and sexual behaviour are also influenced by the production environment and by prior social contact with females (Flowers, 1997). Boars isolated from sows or gilts during their sexual development achieve fewer copulations, display less courting behavior and have fewer ejaculations, although testosterone production is not affected (Hemsworth et al., 1981). Likewise, for mature boars, housing in proximity to sows or gilts improves their sexual behavior (Hemsworth et al., 1981). Considering the importance of the boar's behavior in ensuring a good mating response in the female, this could enhance conception rates and potential litter sizes. The influence of the presence of the sow or gilt on boar behavior is also worth noting for boars reared and maintained in boar studs. Efforts to provide some of the stimulus, particularly pheromonal, associated with sows or gilts can be advantageous in obtaining maximum response and sperm production, particularly from young boars.

2.11.4 Human-animal interactions

Boars that are gently handled have larger testicles and achieve their first copulation earlier than boars handled poorly (Hemsworth et al., 1986). Pigs are able to differentiate between people, either by some nonspecific cue such as the coveralls or slight changes in smell, or by specific personal cues. Therefore, whenever possible,

necessary aversive activities such as vaccinations or tusk trimming should be done by someone other than the usual stockperson or when wearing clothing not routinely worn during pleasant stockperson-animal interactions (Loula, 1997).

Attention to this type of detail helps ensure that the person present during critical stages of reproduction maintains the animal's trust and is not likely to stimulate fearful responses. Long term avers handling results in elevated corticosteroids, reductions to many performance parameters, increased avoidance and difficulty in handling.

2.11.5 Heat detection and mating

The importance of accurate estrus detection and correct time of insemination is undeniable. Boar exposure, particularly direct contact, as well as positive sow-sow interaction shortens the weaning to estrus interval, enhances the LH surge and ovulation and increases the likelihood of conception. The mature boar elicits a very strong 'standing' reflex in the estrus sow. This initial response is generally followed within 20-30 minutes by a refractory or recovery period during which the sow will not respond to further boar stimulation (Loula, 1997). It may take a sow, even one in mid-estrus, in excess of one hour before she can respond positively again to the boar's sexual stimulation. This underscores the importance of supervised, brief intense boar exposure in estrus detection. Prolonged exposure is counterproductive.

The mating environment also influences reproductive success. Ensuring adequate courtship or boar stimulation at mating, whether natural or artificial, is critical to optimizing conception, pregnancy rate and litter size. The most prolific sows are comfortable with their surroundings, properly courted or stimulated and

respond with a complete mating repertoire. Not ensuring a natural mating response in the sow or rough handling around the time of mating, disrupts the normal hormone balance and movement of sperm, eggs and early embryos within the reproductive tract.

Unsupervised mating can reduce reproductive performance, especially when boars are rough or overly aggressive following copulation or if sows become overly aggressive following mating, thereby reducing the boar's future sex drives.

2.11.6 Mating environment

Boars generally show a greater degree of courtship behavior in a designated breeding pen and significant improvements in farrowing rates and litter sizes have been realized by using an appropriately designed pen for breeding (Hemsworth et al., 1991).

2.11.7 Temperature and Photoperiod

The detrimental effects of high ambient temperature and heat stress on reproductive performance are well known. The impact may be direct as a result of increased body temperature or compensatory changes in blood flow or it may be indirect through the hypothalamus involving changes in appetite/feed intake and body metabolism. In boars, decreased sperm motility, increased sperm abnormalities, decreased sperm output and lower libidos are all associated with high temperatures and heat stress. Much of this relates to the requirement for the testes to be maintained below body temperature. It will take 5 weeks, the time for new sperm production, after the heat stress for semen quality to return to normal. Libido returns much sooner but, is not an indication of semen quality (Connor and Orzechowski, www. 2012).

Short photoperiods enhance sperm production and semen quality, it importance for semen collection and preservation for AI (artificial intelligence).

2.11.8 Collection of semen, dilution and processing

Although automated semen collection systems have been developed, semen is mostly collected by the gloved hand technique from a boar trained to mount a dummy sow (Barrabes Aneas et al., 2008). Dummy sows should be solid in construction without sharp edges, and located in a quiet designated semen collection room with a non-slippery floor. A pre-warmed (38ºC) collection container is used. The top of the container is covered with cheesecloth to filter out gel portion of the semen.

The end of the penis is grabbed firmly with a gloved hand and the collection process is initiated with firm pressure to the spiral end of penis with the hand so that the penis cannot rotate. This process imitates the pressure applied by the corkscrew shape of the sow's vagina. Polyvinyl gloves can be used, not latex gloves as these are toxic for the semen (Ko et al., 1989). The first part of the ejaculate (pre sperm) should be discarded. It is clear, watery fluid and does not contain sperm (~25 mL), but it may have a high bacteria count. The sperm-rich fraction should be collected (40-100 mL). It is very chalky in appearance and contains 80-90% of all sperm cells in the ejaculate. Once the sperm-rich fraction is complete, the remainder of the ejaculate is again clearer, watery fluid, and should not be collected (70-300 mL). After collection, the filter with gel should be discarded, and the collection container should be placed in warm water.

The semen should be extended within 15 min. after collection. The ejaculation lasts up to 5 to 8 min, but may continue up to 15 min. About 100 to 300

mL of semen is collected. Semen collection from boars is performed approximately 2 times per week (Vyt et al., 2007). The extension process should be done in a warm room with clean and sterile equipment. The extender is added to the semen, and cold shock should be avoided by diminishing the temperature gradually. A normal ejaculate usually contains enough sperm to inseminate 15 to 25 sows using conventional artificial insemination. Each dose should contain 2-3 billion spermatozoa in 80-100 mL.

2.12 Semen Evaluation

Semen must be evaluated as soon as possible after collection, because changes in temperature, exposure to light and exposure to any type of chemicals, lubricants etc. can change sperm motility and adversely affect fertility.

2.12.1 Motility

Motility should be examined as soon as possible, as motility is the most influenced parameter in the semen analysis. Motile sperm cells will try to swim upward and dead cells will settle to the bottom. The swirling pattern will definitely indicate that there are cells alive. The swirling looks like currents and eddies (Table. 2.5)

Table 2.5 Scoring system for the rate of progression of bovine spermatozoa.

Rating	Microscopic appearance
0	no sperm movement
1	slight tail undulation without forward motion
2	slow tail undulation with slow or stop and start forward motion
3	forward progression at a moderate speed
4	rapid forward progression
5	very rapid progression in which cells are difficult to follow visually

Source: Brahman, www. 2012

2.12.2 Individual motility

Individual motility checks for the progressive movement of the sperm cells. Make the sample by placing a drop of diluents (saline or Na citrate) on a warm slide. Examine the sample quickly as the motility changes very rapidly with heat, light and cold. The motility is a very subjective measurement and is affected by many things, such as diluent (it may be old and hypertonic etc.), cold, the glassware, urine, soap, prostatic fluid, seminal pH, and ion composition.

Table 2.6 Boar semen mass activity rating system.

Rating	Microscopic appearance
0	no swirl – nil or sporadic oscillation of individual sperm
1	no swirl - generalized oscillation of individual sperm only
2	very slow distinct swirl
3	slow distinct swirl
4	moderately fast distinct swirl
5	fast distinct swirl - appearance of good quality ram semen

Source: Brahman, www. 2012

2.12.3 Sperm morphology

Morphology is usually examined with an eosin-nigrosin (Society for Theriogenology) stain (background stain) to highlight the cells. The slide is made by painting a drop or line of stain on a warm slide and then stick to place a small amount of semen into the stain; the semen and stain are mixed using another slide and then slowly push the second slide through the stain and across the first slide while pressing firmly down (Fig. 2.5). The goal is to get a dark background, as the stain is a background stain and is not intended to stain the cells. In fact, some cells will stain red. Examine the cells under 1000X (oil) to fully assess the morphology. Count 100 cells and, in a practice situation. Using eosin-nigrosin all the cells will appear flat, as if looking at your hand. In order to do a full spermiogram, differentiate the abnormalities by type. A phase contrast microscope can be used. A phase contrast mount is made by placing a small amount of semen in formal-buffered saline to kill

and preserve the cells. Then place a drop of the formal-buffered saline sample onto a slide and place a cover slip over the drop.

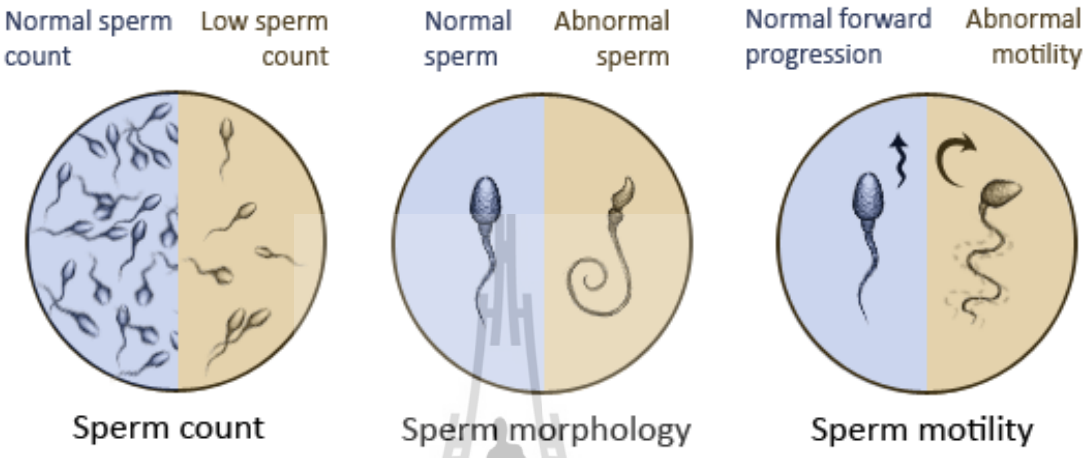

Figure 2.6 Comparison normal and abnormal on count, morphology, motility of sperm.

Source: http://www.fertilaidformen.com/how-to-increase-sperm-count

2.12.4 Abnormalities

Abnormalities are classified as primary and secondary. Primary abnormalities are thought to arise in the testes, whereas secondary abnormalities arise in the epididymis or ejaculate. Secondary abnormalities may be just as serious primary. Primaries abnormalities decrease through transit and secondary abnormalities increase through transit. Major and minor may be a better classification.

Major problems cause Early Embryonic Death (the pregnancy was lost after the pregnancy recognition signal) or prevent fertilization. For example, an acrosome problem prevents zona entry and a nuclear problem leads to non fertilization. Minor problems such as tail abnormalities etc. stop sperm movement, so the sperm cell cannot get to egg.

2.12.5 Abnormalities of the sperm cells

Decapitated sperm (not pictured), the basal plate is defective; tails move (wrap around drops); 100% cells involved; occurs in epididymis (secondary) (Brahman, www. 2012):

1. Loose heads, these may be increased on the first ejaculate (rusty load) because the tail attachment is frail.
2. Knobbed or flat acrosome (not pictured), the acrosome folds over itself of the apex of the acrosome is knobbed or flattened. When 20% of the cells have acrosome problems the result may be infertility in the bull. This condition may be hereditary in Charolais, Hereford, and Holsteins.
3. Wrinkled acrosome, this may reflect a nuclear problem which prevents zona attachment by the sperm cell. It is a rare condition.
4. Pyriform and tapered heads, the nuclear material is poorly distributed. The defect may be subtle.
5. Giant or small heads, this nuclear problem. If the head is twice normal size the cell is a giant cell.
6. Nuclear vacuoles, these distort the shape of the head.
7. Diadem defect, invaginations in the nucleus, mostly by the post nuclear cap. The pit lacks DNA. The condition may be associated with stress in bulls and may come and go as stress changes.
8. Dense proximal droplets, this arises in the epididymis and indicates maturation problem.
9. Stump tail defect (not pictured) - this is an axonemal problem. It looks

like a cytoplasmic drop and has a poor prognosis. The incidence increases with age.

10. Midpiece defects, see lumps on the midpiece that can be confused with proximal drops.
11. Coiled midpiece (not pictured), this is an epididymal defect, but is a major defect. The midpiece problems (not Dag).
12. Abaxial midpiece, the implantation fossa is defective. There is generally a low incidence and fertility is not affected; (normal in stallion).
13. Coiled mainpiece, the mainpiece is coiled within the plasma membrane.
14. Abaxial midpiece, the implantation fossa is defective. You may see abaxial or double midbpieces, there is generally a low incidence and fertility is not affected; (normal in stallion).
15. Coiled mainpiece, the mainpiece is coiled within the plasma membrane
16. Teratospermia, the entire cell is degenerative
17. Bent tails, the bend in the tail may include a droplet which may be in the membrane.
18. Physiologic (distal) droplet, some consider this a minor defect, but in fact it may be a major defect. These cells do not freeze well because the water in droplet crystalizes and ruptures the cell membrane.

Table 2.7 Overview of the cut-off values for porcine semen quality in artificial insemination.

Semen parameter	**Requirement (%)**
Motility	<60
Abnormal morphology	
Normal acrosomes	
Cytoplasmic droplets	
Proximal droplets	<20
Distal droplets	
Coiled tails	
Primary abnormalities	<10
Secondary abnormalities	<20

Source: Britt et al., 1999

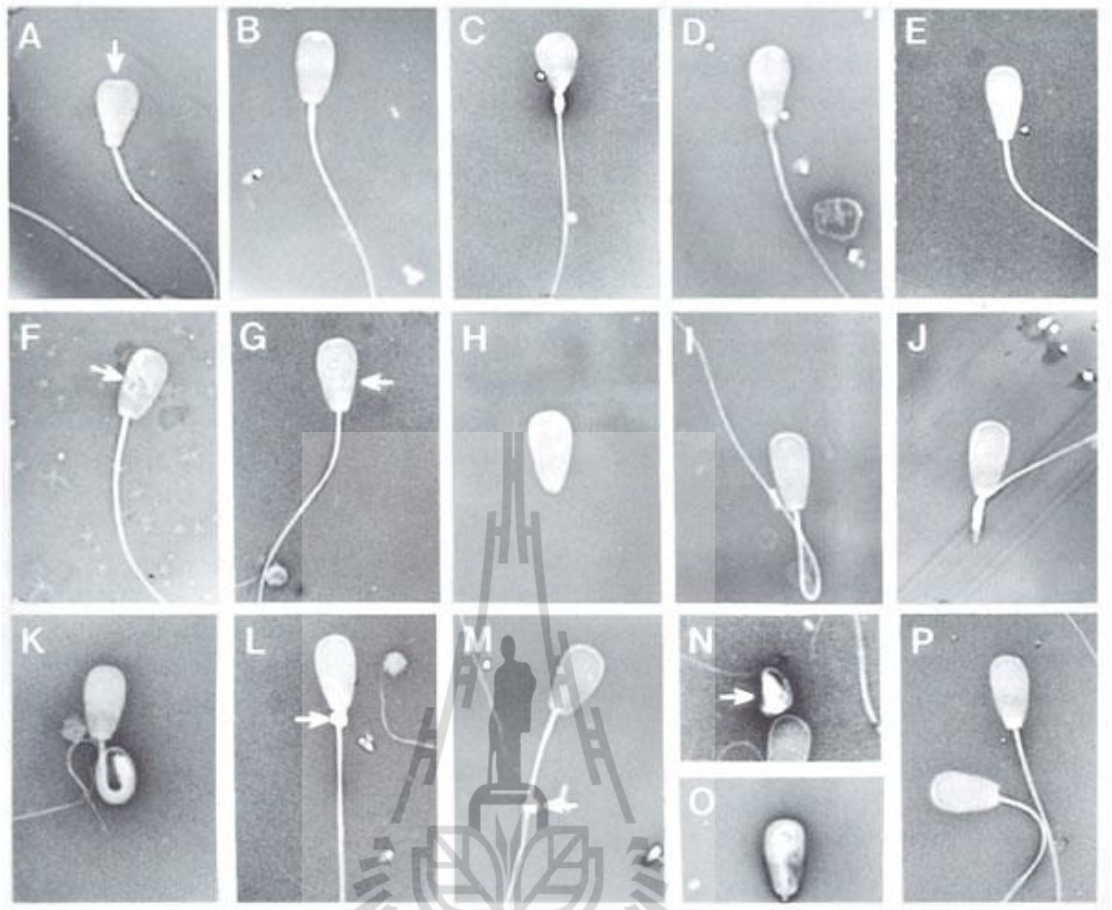

Figure 2.7. Common sperm abnormalities. A. Knobbed acrosome (common form), B. Knobbed acrosome (beaded form), C. Pyriform head (severe), D. Pyriform head (moderate), E. Pyriform head (slight), F. Nuclear vacuoles, G. Diadem defects, H. Detached head, I. Distal reflex, J. Dag-like defect (broken midpiece), K. Dag-like defect (severely bent midpiece), L. Proximal droplet, M. Distal droplet, N. eratoid (severe), O. Teratoid (moderate), P. Normal spermatozoa.

Source: Brahman, www. 2012

2.13 Trace minerals in sexually active boars

In 1998, the National Research Council (NRC) published the most recent Nutrient Requirements of Swine. Table 2.8 shows are the NRC requirements for some trace minerals in breeding boars. With regard to Se, it should be noted that current U.S. Food and Drug Administration (FDA) regulations allow up to 0.3 ppm of added Se in the diets of all pigs.

Table 2.8 Trace mineral requirements of sexually active boars (NRC, 1998).

Feed intake	**2.0 kg/d Diet requirement (ppm)**
Copper	5
Iodine	0.14
Iron	80
Manganese	20
Selenium	0.15
Zinc	50

Source: NRC, 1998

2.14 Selenium and boar productivity

Se is essential for the normal development of spermatozoa and is incorporated into the mitochondrial capsule protein. Se is also a component of GPx, an enzyme that protects cellular components against free radicals and is an antioxidant for cellular membrane lipids (Hansen and Deguchi, 1996). Generally, research has shown that Se supplementation in boars increased GPx activity and Se concentrations in whole

semen, spermatozoa, and seminal plasma, as well as in the circulation, kidney, liver, heart, skeletal muscle, testis, epididymis, prostate, seminal vesicle, and bulbourethral gland (Marin-Guzman et al., 1997; Segerson et al., 1981). However, Kolodziej and Jacyno (2004) reported that addition of Se increased its concentration Se, but decreased GPx activity, in seminal plasma. The amounts of Se in seminal plasma of boars receiving either organic Se or inorganic Se were similar however GPx activity favored the inorganic Se source (Jacyno et al., 2002).

In general, Se supplementation had little effect on the growth performance of boars. Indeed, growth rates, feed intakes, feed conversion efficiencies, and testicular sizes were similar for control boars and boars provided extra Se in the diet (0.5 ppm; Kolodziej and Jacyno, 2004; Marin-Guzman, 1997) or via s.c. injections at 14-d intervals (Segerson et al., 1981).

In contrast to these reports, Henson et al. (1983) reported that boars fed diets supplemented with Se exhibited some signs of retarded growth and sexual development. In that study, boars were fed a basal corn and soybean meal diet (0.05 ppm Se) or the basal diet supplemented with a Se premix at concentrations of either 0.10 (n = 11) or 0.25 ppm (n = 11). Experimental diets were fed on an ad libitum basis from 54 d of age until approximately 6 mo of age, and were then limit fed at a rate of 2.27 kg/boar/day. Treatment by age interactions existed for BW, testis width, libido (subjectively scored twice weekly upon exposure to ovariectomized, estrogen-treated gilts), and plasma testosterone levels, and in general values were greater for boars fed the basal diet than for those fed the basal diet supplemented with Se.

Jacyno et al. (2002) conducted an experimental boars of diets supplement with either 0.2 ppm Se-yeast and 60 ppm vitamin E (n = 40), or 0.2 ppm inorganic Se (Na_2SeO_3) and 30 ppm vitamin E (n = 40). Source of Se was confounded with

concentration of vitamin E fed. The study was conducted from 70 to 180 day of age in both the summer and winter seasons, and the daily feed ration was gradually increased along with increasing body weight (BW). Compared with boars that received the organic Se, boars receiving the inorganic Se had higher average daily gain and better feed conversion efficiency, effects that were most pronounced during the winter. There were no effects of treatment on leanness or testicular size.

Effects of Se supplementation on reproductive performance in breeding age boars. Several studies have been conducted to evaluate the effect of Se supplementation on reproductive characteristics in breeding boars (Segerson et al. 1981; Marin-Guzman et al., 1997, 2000a, 2000b; Jacyno, 2002; Kolodziej and Jacyno, 2004).

Segerson et al. (1981) conducted an experiment during which crossbred boars were fed a low Se diet (0.025 ppm) made of corn starch and yeast on an ad libitum basis beginning at 78 day of age. Boars received subcutaneous injections of sodium selenite (0.33 mg/kg BW; n = 4) or 0.9% saline (n = 5) at 14-d intervals. At 230 day of age, boars were exposed to estrogen-treated, ovariectomized gilts and ejaculates were collected at 4- to 6-day intervals until a total of four ejaculates had been obtained from each boar. Injections of Se increased the number of spermatozoa/ejaculate but had no effects on the percentages of viable or morphologically normal spermatozoa.

In a study involving 192 Landrace x Yorkshire x Duroc boars, Marin-Guzman et al. (1997) investigated the effects of dietary Se and vitamin E supplementation on reproductive function. From weaning through 18 mo of age, boars were fed a basal diet made of yeast and dextrose or cornstarch (0.067 ppm Se), the basal diet supplemented with Na_2SeO_3 (0.5 ppm), the basal diet supplemented with vitamin E

(220 IU/kg of diet), or the basal diet supplemented with both Se (0.5 ppm) and vitamin E (220 IU/kg of diet). Diets were consumed on an ad libitum basis from weaning to approximately 145 kg BW and thereafter were limit fed to individual boars at a rate of 2 kg/day.

In general, both dietary Se and vitamin E enhanced boar semen quality, but the positive effects of added Se on semen characteristics were more pronounced than were the effects of added vitamin E. During a 16-week experimental period that began when boars were approximately 9 month of age, semen was collected three times weekly. There were no effects of treatment on semen volume, sperm concentration or total spermatozoa. Sperm motility remained relatively constant when the diet was fortified with Se but declined in boars fed the unfortified Se diet.

The percentage of normal spermatozoa declined in all treatment groups over the 16-week period, but this decrease was least pronounced in boars receiving selenium supplementation. Boars fed the unfortified Se diet had the highest percentages of spermatozoa with cytoplasmic droplets and bent and shoe hook tails. Subsequently, semen was collected twice weekly for 8 week and at the end of this period, semen quality was again assessed. Supplementation with Se increased semen volume and the percentages of motile and morphologically normal spermatozoa. Gilts were artificially inseminated with semen from experimental boars and were killed 5 to 7 day later. Se supplementation increased fertilization rates and the number of accessory spermatozoa.

In other reports, histological examination of testicular tissue of 18 month-old boars employed in this study revealed that supplementation with Se, but not vitamin E, increased the number of Sertoli cells, round spermatids, and secondary spermatocytes (Marin-Guzman et al., 2000a; 2000b),. Marin-Guzman et al. (2000a) suggested that Se

has a role in establishing the number of Sertoli cells and boar spermatozoal reserves. Also, Se deficient boars produced spermatozoa with decreased ATP concentrations, and electron microscopy revealed that these cells had structural abnormalities to the tail midpiece, including altered mitochondrial shape and orientation and poor contact of the plasma membrane to the helical coil.

Jacyno et al. (2002) performed an experiment the objective of which was to compare organic versus inorganic Se supplementation on semen characteristics in boars fed during either the winter or summer. In pigs, organic Se is more effectively retained than inorganic Se (Mahan and Parrett, 1996).

Boars were fed diets supplemented with either 0.2 ppm Se-yeast and 60 ppm vitamin E (n = 40), or 0.2 ppm inorganic Se (sodium selenite) and 30 ppm vitamin E (n = 40) beginning at 70 days of age. Beginning at 180 days of age, boars were trained to mount an artificial sow for semen collection. There was no effect of treatment on ejaculate volume or the percentage of motile spermatozoa.

In contrast, the concentration of spermatozoa and total spermatozoa were higher in boars fed the diet containing the organic source of Se. The quality of spermatozoa was also enhanced in boars receiving the organic Se source. Indeed, boars fed the diet containing organic Se had higher percentages of spermatozoa with normal acrosomes and that passed a hypoosmotic swelling test, and lower percentages of spermatozoa with minor or major morphological abnormalities.

Beneficial effects of the organic Se source were most evident in boars fed during the winter. Although these data are consistent with the notion that supplementation of diets with an organic source of Se is superior to supplementation with inorganic Se, source of Se in this study was confounded with concentration of vitamin E.

Kolodziej and Jacyno (2004) evaluated semen characteristics in boars fed diets containing either 0.2 ppm Se and 30 ppm vitamin E (n = 20), or 0.5 ppm Se and 60 ppm vitamin E (n = 20), beginning at 70 day of age. Beginning at 180 day of age, boars were trained to mount an artificial sow and allowed semen collection. There was no effect of treatment on gel-free semen volume or the percentage of motile spermatozoa.

In contrast, the concentration of spermatozoa and total spermatozoa were higher in boars fed the diet containing the higher concentration of Se and vitamin E. Boars fed the diet containing the higher concentration of Se and vitamin E had higher percentages of spermatozoa with normal acrosomes and that passed a hypoosmotic swelling test, and lower percentages of spermatozoa with minor or major morphological abnormalities. While positive effects of a higher concentration of both dietary Se and vitamin E were demonstrated, the relative contribution of each substance toward enhanced semen quality cannot be ascertained.

Few studies have evaluated the effects of trace mineral and vitamin supplementation on libido in boars. It is noteworthy that Kolodziej and Jacyno (2004) reported no effects of the higher concentration of Se and vitamin E on the number of mounts of the artificial sow, the period from entering the collection area to the start of ejaculation, or the duration of ejaculation. In concert with these findings, Segerson et al. (1981) found no differences in the concentration of testosterone in serum of breeding boars of different Se statuses. In contrast, boars fed a diet containing 0.2 ppm organic Se and 60 ppm vitamin E mounted the artificial sow and began ejaculating more quickly, and had longer durations of ejaculations, than boars fed a diet containing 0.2 ppm inorganic Se and 30 ppm vitamin E (Jacyno et al., 2002).

Se deficiency symptoms continue to be reported in boar, including abnormal sperm cells in boars. Se deficiency may be more common when Na_2SeO_3 serves as the major source of total dietary Se, because Na_2SeO_3 s is not stored in body tissues of pigs as well as Se found in grains or grain by-products. That mean, grains incorporate Se in an organic form, primarily as Se-met. Grains grown in Se-deficient soils have lower organic Se content. Se-yeast, which contains Se-met was approved for use in swine diets in July 2002. Legal limits for Se addition to swine feeds remain at 0.30 ppm. However using a more biologically active source of Se may prevent deficiency symptoms from occurring even when the same levels of total Se are supplemented in the diet.

The studies published over the last years focus mainly on organic compounds of Se and their application in the supplementation of livestock feeding. For monogastric animals, Se is available both in the form of organic (seleno-amino acids) and inorganic compounds (Na_2SeO_3, Na_2SeO_4); however, the availability of organic compounds as well as their biological efficiency are significantly higher than those of inorganic Se compounds. Organic Se retention in pigs was by 85% higher than that of inorganic Se (Mahan and Parrett, 1996).

According to Pehrson (1994) supplementation with organic forms of Se needs an additional portion of vitamin E. Little information can be found in the available literature on the influence of organic Se compounds on the reproduction of pigs. Studies on boars have demonstrated that organic Se is much more positive to reproduction of the males compared with an inorganic form of this element (Mahan et al., 2002). High concentration of Se in testes and epididymides of the boar implies that this element is essential for the process of production and maturation of spermatozoa. Se is a component of mitochondrial capsule selenoprotein, which maintains the

stability of spermatozoa mitochondrial. Studies carried out on male belonging to various species of the farm animal have shown the positive influence of Se of semen quality, especially on the concentration, vitality, mobility and morphological defect of spermatozoa (Marin et al., 1997, Saaranen et al., 1989).

On the contrary, other studies demonstrated that an addition of Se to diet did not improve the semen quality of rams (Buchanan et al., 1969), bulls (Bartle et al., 1980, Segerson and Johnson 1980) or boar (Segerson et al., 1981b, Henson et al., 1983). Probably, Se was not deficient in the animal in those students and therefore no influence was observed of element on the reproductive processes of the male (Heimann et al., 1984).

Bioavailability of Se depends on the species of animal, the source, level and on which chemical from of the element is present in the diet. In monogastric animal, organic Se (seleno-amino acid) is more effectively retainedd than inorganic Se (Na_2SeO_3, Na_2SeO_4) that is commonly used in animal diets. Mahan and Parrett (1996) found that in pigs receiving 0.3 ppm inorganic Se. The results obtained by the authors showed that the amount of Se retented in the body of pigs receiving 0.3 ppm inorganic Se was equivalent to that at 0.13 ppm organic Se in diet.

2.15 Effect of Se in pig hair color

The Se content in swine hair was affected by its color (Wahlstrom et al., 1984). Hair Se concentration has also been shown to increase in pigs as the dietary level of Se increasing (Goehring et al., 1984). Hair mineral compositions can be affected by the nutrient content of circulating extracellular fluids indicated that secretions of the sebaceous and apocrine glands contain minerals that adsorb to hair. It

has been suggested that hair analysis might provide meaningful information about an animal's mineral (Kim and Mahan, 2001b).

Selenosis begins after growing-finishing pigs consume dietary Se levels from 5 to 7 ppm Se and the condition becomes increasingly exacerbated and occurs sooner as the dietary level increases. Selenosis can be precipitated by using either inorganic or organic Se source. When dietary levels are > 5 ppm Se, the selenosis effect seems to be greater when inorganic Se is fed (Kim and Mahan, 2001b). Tissue Se concentrations increase when high dietary Se levels are provided, but the liver hair and hoof accumulate greater Se concentrations than other body tissues. Hoof separation and alopecia are two signs of Se toxicity and the damage to these tissues may be because of their higher Se concentrations.

When white-haired pigs consume high dietary levels of Se, alopecia occurred sooner than when pigs are of a dark hair color (Kim and Mahan, 2001b). This effect occurs in the white hair body area even when both hair colors are present on the same pig. Dark hair seems to retain more Se and less dark body hair was lost, but only when the dietary Se level was > 3 ppm. Hair Se concentration between white or dark colors therefore differs when dietary levels are > 3.0 ppm but does not seem to differ between these hair colors when dietary Se levels are < 1.0 ppm. Because the white-haired animals in this experiment responded more severely and sooner to high levels of dietary Se, the results suggest that hair Se may be an avenue of ridding the body of excess Se and that white-haired pigs are less capable of tolerating excess Se. White haired pigs had higher circulating blood Se concentrations and showed earlier evidence of the selenosis condition.

Hair Se is not expected to be reutilized by the body and can thus be considered as metabolically lost once deposited in that tissue. Se that is not deposited in tissues and not eliminated in the excretory routes may thus contribute to the more rapid onset of alopecia. The results suggest that pigs may differ in their ability to retain Se in hair tissue which may therefore influence their response to the selenosis condition. However, demonstrated that hair Se did not differ by color when the dietary Se level was < 1.0 ppm and in fact declined when 0.30 ppm Se was fed. An exception to that conclusion was the black hair on the Hampshire breed, whose hair Se concentration was lower than that of the red or white hair of the other two breeds. It is possible that genotypes may differ in their Se requirements as suggested by Stowe and Miller (1985).

2.16 Effect of Se in spermatozoa

A biochemical role for Se in animals was established by Rotruck et al. (1983) with the finding that Se is an integral structural component of the mammalian selenoenzyme, GPx (GSH-Px). It is known that mammalian spermatozoa have a high content of polyunsaturated fatty acids on their cell membrane. This characteristic renders spermatozoa particularly prone to the deleterious effects of reactive oxygen species, and in fact much of the reactive oxygen species-induced damage is a result of spermatozoa membrane lipid peroxidation (Aitken et al., 1989).

2.17 Se metabolism in animals

Se supplementation is known to affect the antioxidant defenses of chicken semen (Surai et al., 1998). Furthermore, Edens (2002) showed that, when cockerels

were fed on a basal diet containing 0.28 ppm Se without additional dietary supplementation of this trace element, the percentage of normal spermatozoa was only 57.9% and two major abnormalities seen were bent midpiece (18.7%) and corkscrew head (15.4%). When this diet was supplemented with an additional 0.2 ppm Se in the form of selenite, the percentage of normal spermatozoa increased to 89.4% and abnormalities in the form of bent midpiece and corkscrew head were decreased down to 6.2 and 1.8%, respectively. However, when organic Se was included in the cockerel's diet in the same amount, semen quality was further improved and those abnormalities decreased down to 0.7 and 0.2% and the percentage of normal spermatozoa increased up to 98.7%.

These results clearly showed that the form of dietary Se supplementation is a crucial factor of its efficiency, with organic Se being much more effective in comparison to inorganic Se. Se deficiency is associated with midpiece damage to spermatozoa, it is clear that the midpiece of spermatozoa of the Se deficient male is broken. In such conditions, sperm motility and fertilizing capacity would be compromised. Organic selenium can also improve fertility and, more importantly, increase the duration of fertility (Agate et al., 2000). Thus, avian spermatozoa might be expected to have systems which will maintain stability throughout this period.

Metabolic pathway for Se metabolism in animals has been presented by Ip (1998) and the seleno-compounds in animal tissue have been summarized by Whanger (2002). The metabolic pathways for Se in animals are shown in Fig. 2.7. Organic Se such as Se-met or inorganic Se can be converted to a common intermediate, Hydrogen selenide (H_2Se).

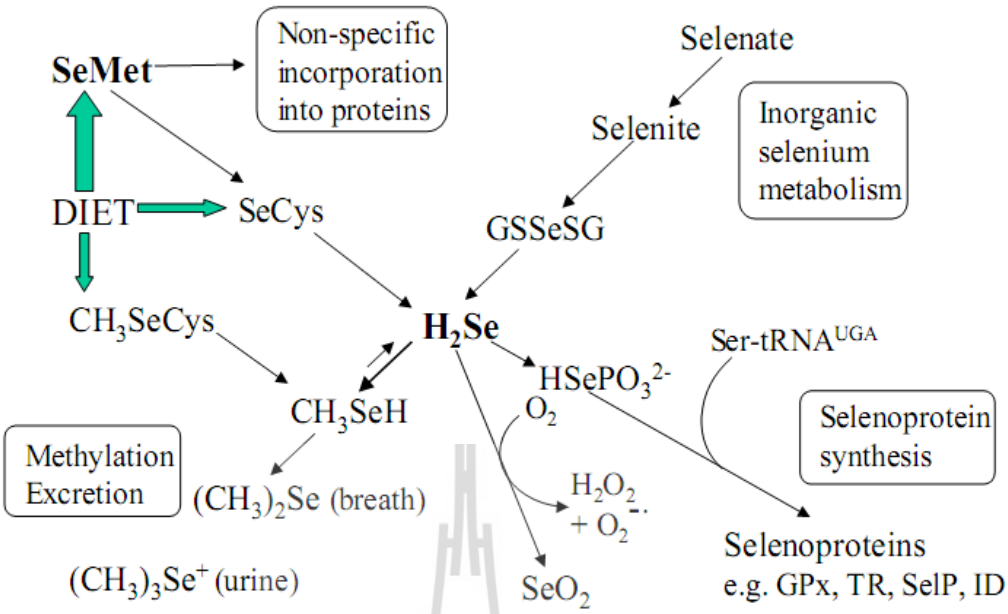

Figure 2.8 Metabolic fate of selenomethionine and other organic selenium compounds from the diet (adapted from Combs, 2001)

Ingested Se-met from selenium-yeast is absorbed in the small intestine. Se-met from selenium-yeast and food proteins can be incorporated non-specifically into proteins such as albumin and haemoglobin in place of methionine. Alternatively it can be trans selenated to selenocysteine (Se-Cys) which is then converted to hydrogen selenide (H_2Se) by a β-lyase. The H2Se formed may be converted to selenophosphate (HSePO32) by selenophosphate synthetase. Selenophosphate reacts with tRNA-bound serinyl residues to give Se-Cys bound tRNA from which selenocysteine is incorporated in selenoproteins (Berry et al., 1991, 1993).

2.18 Summary of Se usage

Although research reports focusing on trace mineral supplementation of the sexually active boar are scarce, some general conclusions can be drawn. A prolonged period of restricted dietary zinc negatively impacts leydig cell morphology and probably secretion of testosterone and limited research suggests that sperm produced per ejaculate is optimized at zinc concentrations between 80 and 150 ppm. With regard to reproductive performance, there is strong evidence to support the addition of Se to boar diets. Improvements in sperm production, sperm morphology and actual fertility have been reported for boars fed diets supplemented with Se generally at levels of 0.5 ppm.

The FDA allows a maximum of only 0.3 ppm supplemental Se in the diet of swine. Evidence suggests that organic sources of Se may be more bio-available than inorganic Se. Considering this, further research investigating the effects of organic source Se on reproductive function in boars is warranted.

2.19 Selenium yeast

Se supplements available in order of increasing cost include: the inorganic forms, i.e. sodium selenite, sodium hydrogen selenite and sodium selenate; organic forms, i.e. Se-yeast and the seleno-amino acid, Se-Met, the major Se species in Se-yeast. The replacement of Met by Se-met as a rule does not significantly alter protein structure but may influence the activity of enzymes if Se-met replaces Met in the vicinity of the active site. Because the CH_3-Se group of Se-met is more hydrophobic than the CH_3-S-moiety of Met, substrate access may be affected, altering the kinetic parameters (Gerhard, 2000).

Under appropriate conditions, yeasts are capable of accumulating large amounts of trace elements such as Se and incorporating them into organic compounds (Suhajda et al., 2000). This mineral is absorbed during the yeast growth process. Yeast is known for its high protein content and therefore, more Se can be incorporated by replacement of sulphur in proteins compared with Se from plant sources (Fig 2.8). The Saccharomyces clade is composed of seven species: *S. bayanus, S. cariocanus, S. cerev isiae, S. kudriavzevii, S. mikatae, S. paradoxus* and *S. pastorianus*. Among these species, *S. cerevisiae* and *S. bayanus* are known for their role in alcoholic fermentation. *S. bayanus* has some specific properties: cryotolerance, production of smaller amounts of acetic acid and ethanol, but higher amounts of glycerol and succinic acid (Christine Le Jeune et al., 2007).

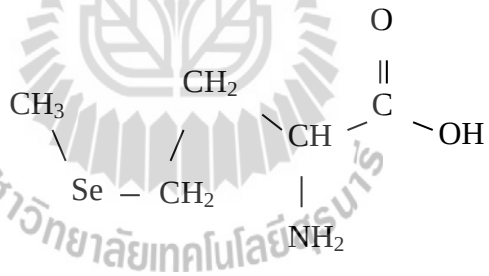

Figure 2.9 Chemical structure of Se-met ($C_5H_{11}NO_2Se$).

2.20 Manufacture and quality control of selenium-enriched yeast production

Se-yeast is the product of the aerobic fermentation of *S. cerevisiae* in a Se-enriched medium. Different companies use different strains of *S. cerevesiae* and may describe them either as bakers or brewer's yeast. The medium is usually beet or cane molasses to which are added vitamins, nutritional salts and other growth factors to

ensure maximal biomass, and measured amounts of Se salts (for example, sodium selenite) as the Se source. Control of pH, temperature, Se feeding profile and aeration allows optimal growth of the yeast strain and maximum biomass production. A Se-yeast cream is produced that is then pasteurised thereby killing the yeast, and dried, frequently by spray drying.

As a result of the fermentation in the Se-enriched medium, the Se becomes organically bound to the yeast. The amount bound should be greater than 90%, typically 94% (percentage of complexed organic Se found in three lots of LAMINe Se-yeast by KABS Laboratories, St Hubert, QC, Canada) in the case of one manufacturer (Lallemand, Montreal, Canada). In this case, Lallemand Research and Development reported that about 83% of the Se in the yeast was bound to yeast proteins, including cell wall proteins (Margaret, 2004).

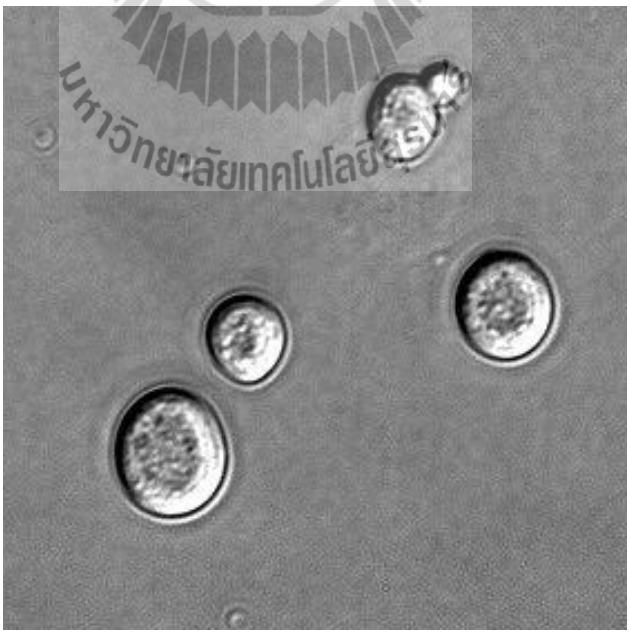

Figure 2.10 Morphology of *S. bayanus*,

Source: Wikipedia, www. 2012

Scientific classification of *S. bayanus*

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Saccharomycotina

Class: Saccharomycetes

Order: Saccharomycetales

Family: Saccharomycetaceae

Genus: Saccharomyces

Species: *S. bayanus*

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Combs, 2008). It is generally believed that the ingestion of the organic Se compounds is better and safe than that of the inorganic Se. Some microorganisms produce biomass with high protein content and meanwhile transform inorganic Se (low bio-availability and potentially toxic) into organic form (safe and highly bioactive). Organic Se complexes and Se containing amino acids are considered to be the most bio-available for human and animals (Zhou et al., 2009).

Under appropriate conditions, yeasts are capable of accumulating large amounts of trace elements such as Se and incorporating them into organic compounds (Suhajda et al., 2000). This mineral is absorbed during the yeast growth process. The ability of *Saccharomyces cerevisiae* to transform inorganic Se into organo-selenium compounds (especially Se-met) is known and it is dependent on growth conditions. Yeast is able to accumulate high concentrations of Se (3 mg/g) (Korhola et al., 1986) and transform inorganic Se mainly into Se-met (Ponce de León et al., 2002; Reyes et al., 2004). Continuous fermentations in optimum medium of sodium selenite (Na_2SeO_3) demonstrated higher biomass and Se content compared to the fermentation with sodium selenate (Na_2SeO_4) (Ali Demirci et al., 1999). Yeast is known for its high protein content and therefore, more Se can be incorporated by replacement of sulphur in proteins compared with Se from plant sources.

Also, the industrial production of yeast is more manageable than the industrial production of Se enriched plants. The *Saccharomyces* clad is composed of seven species: *S. bayanus*, *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus* and *S. pastorianus*. Among these species, *S. cerevisiae* and *S. bayanus* are known for their role in alcoholic fermentation. *S. bayanus* has some specific properties: cryotolerance, production of smaller amounts of acetic acid and ethanol, but higher amounts of glycerol and succinic acid (Christine Le Jeune et al., 2007). The

primary object in this work is to construct a simple and robust control strategy for Se enriched yeast production using single dose addition of sodium selenite.

3.3 Materials and Methods

3.3.1 Materials

Fourteen yeast strains were collected in this study. The DV-10, D254, K1-V1118, Bayanus, ICV D47, 71B-1122 strain were obtained from The LALLEMAND Inc., Montreal Canada (LALVIN). The pasture Red, Pasture Champagne strains were obtained from the Universal Food Corporation Milwaukee (RED-STAR). The CY-3097 strain was obtained from CP Co., Ltd., Thailand . The L3190, NT50, Cosdee Blance strain was obtained from Suranaree Univesity of Technology, Thailand. The beerkit strain was obtained from Auckland New Zealand (BREWTEC). *S. cerevisiae* no.34 obtained from Technical University of Munich (TÜM), Weihenstephan, Germany.

3.3.2 Quantification of Se accumulate in yeast cell

The determination accumulated Se of cell yeast activities of 14 *Saccharomyces* strain cultured under aerobic condition was done as following; 6 ml of modified YM medium (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose, and 10 mg/L (Na_2SeO_3) in 15 mL test tube was incubated at 30 ºC. Growth was monitored by measuring absorbance at 600 nm. Supernatant was stored at 4 ºC for analysis.

3.3.3 Selenium purification

For purification of Se, cell were grown in 1 L flasks containing 500 mL of modified YM medium (10, 20, 30, 40, 50, 60 mg/L Na_2SeO_3) Supernatant solution

was collected by centrifuge at 10,000 rpm (GSA rotor, SORVALL RC5C Plus Super speed Centrifuge) for 5 min. The supernatant obtained after centrifugation was save at 4 ºC for analyze Se

3.3.4 Fermentation condition

The first pre-culture was inoculated with a single colony, cultivated on a rotary shaker at 30 ºC for 24 h. To ensure all yeast preparations started with the same amount of cells, aliquots containing 1×10^7 cell/mL and then inoculated in a 5 L fermentor (Sartorius Biostats, Germany) with initial working volume of 4 L. The agitation speed was set at 200 rpm. Culture temperature was kept at 30ºC. Aeration was maintained at an air working volume per minute (vvm). Inorganic Se was added to the sterile medium before the start of yeast cultivation as a solution of Na_2SeO_3, at a concentration of 20 mg/L Na_2SeO_3 (Zheng Wang et al., 2010).

3.3.5 Analytical Methods

The culture medium was centrifuged at 4,000 rpm for 10 min and washed twice with the deionized water. The cell was dried at 70 ºC with a drying oven for 48 h to obtain dry cell for the estimation of dry cell weight (DCW, mg/ml). At the same time the optical density (OD) of samples was measured spectrophotometrically at 660 nm (Spectronic, Genesys 20, USA). The digestion of the samples (10 mL) was performed with 10 µL HCl (30%) and 50 µL H_2O_2 (30%) at 90 ºC for 1 h. This solution was then filtered through a 0.25 µm nylon membrane filter (Sarorius, minisart, Geramany) and the total Se concentration was determined using Inductively coupled plasma mass spectrometry (ICP-MS, Agilent, USA). The number of yeast cells/mL was determined by counting under a microscope (ECLIPSE E2000, Nikon, Japan).

Table 3.1 Operating condition for total Se determination using ICP-MS.

Parameter	ICP-MS conditions
Reflected power	1550 W
Sampling depth	8 mm
Carrier gas (Argon)	0.85 L/min
Makeup gas (Argon)	0.34 L/min
Nebulizer pump	0.08rps
Temperature	2 ºC

3.4 Results and Discussion

3.4.1 Effect of Na_2SeO_3 single addition on Se accumulation

*S. bayanu*s showed the highest to Se (10 mg/L Na_2SeO_3) accumulation potential. Organic Se content was analysed by ICP-MS for Se concentration against DCW and incorporated Se in Fig 3.1 and 3.2, respectively.

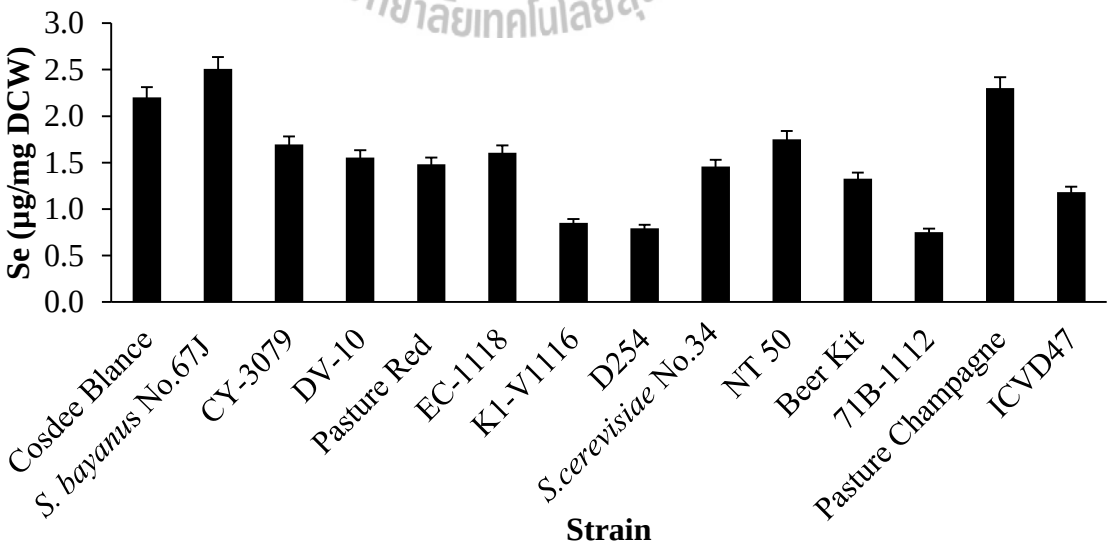

Figure 3.1 Yeast strains were cultured in 10 mg/L Na_2SeO_3 and analyzed the incorporated Se concentration against DCW.

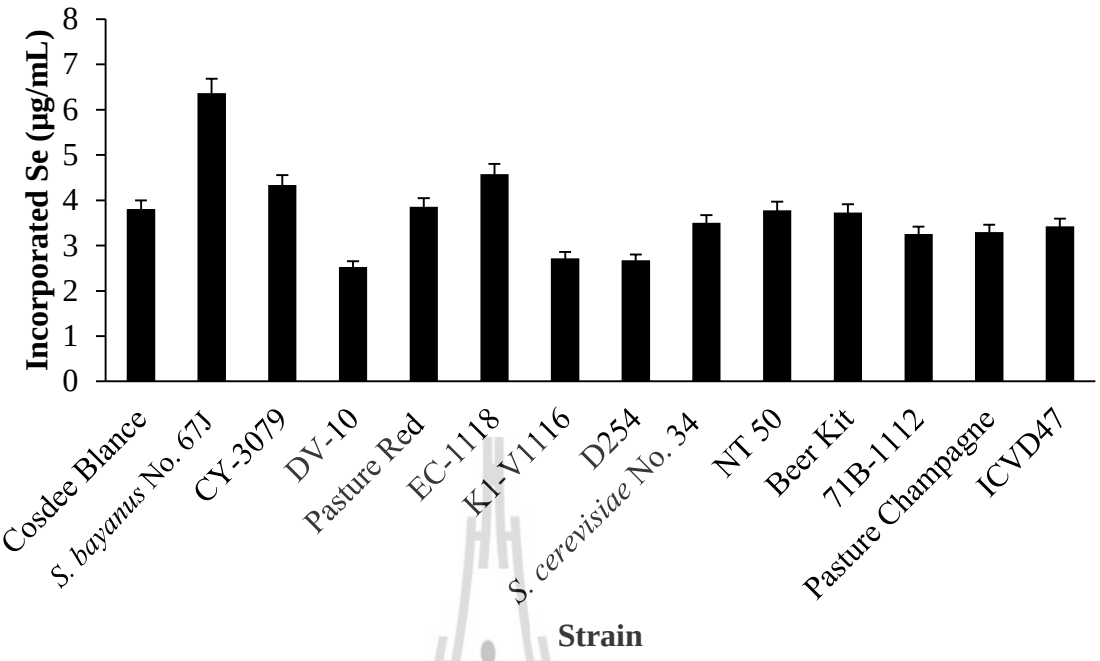

Figure 3.2 Yeast strains were cultured in 10 mg/L Na_2SeO_3 and analyzed the incorporated Se concentration against the number of yeast cell.

S. bayanus cultivated in the media with different concentrations of Se. Increasing of Se concentration from 10 to 60 mg/L diminished specific growth rate from 0.089 to 0.017 h^{-1} (Fig. 3.3). The more Se added to the yeast, the yeast growth was inhibited however, *S. bayanus* showed the highest to Se (20 mg/L Na_2SeO_3) accumulation potential at 48 h. When increase concentration of Se the total yeast was decrease (Fig. 3.4), indicate that Se also causes toxic to growth of yeast, which might be effected to membrane integrity and genotoxicity. (Alicia lzquierdo et al., 2010).

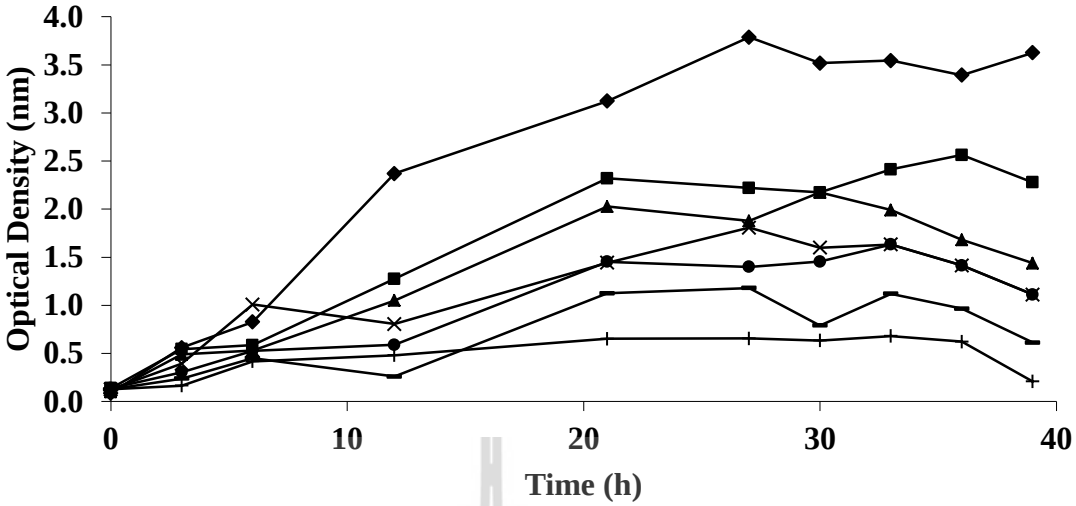

Figure 3.3 Growth of yeast in the media with different concentrations of Se from 0 to 60 mg/L; 0 mg/L (◆), 10 mg/L (■), 20 mg/L (▲), 30 mg/L (×), 40 mg/L (●), 50mg/L (-), 60 mg/L (+).

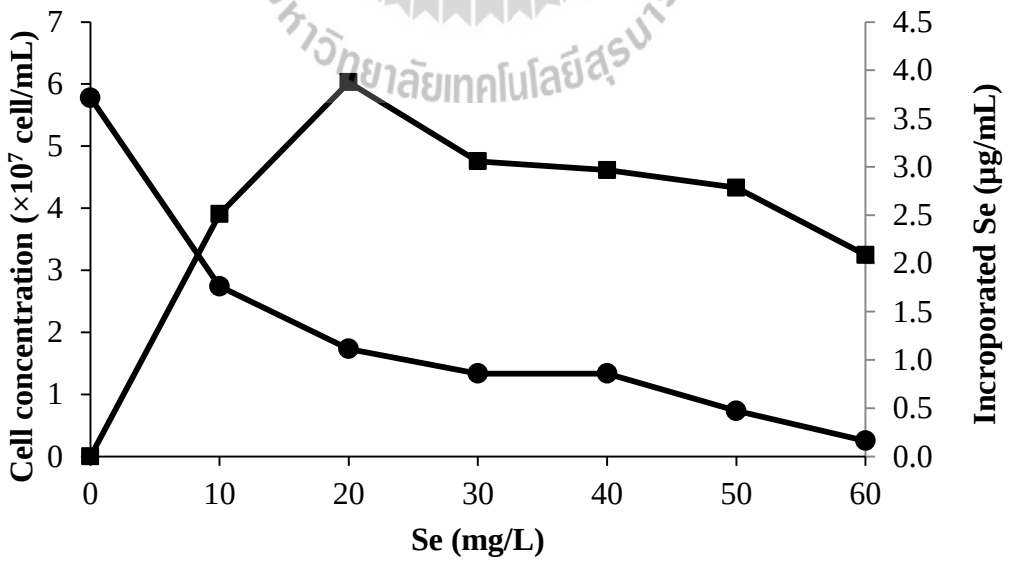

Figure 3.4 The effect of Na_2SeO_3 single addition on Se accumulation in a 5 L fermentor at 48 h: cell concentration (●), incorporated Se (■).

The scale up experiment was carried out in a 5 L fermentor. Na_2SeO_3 at final concentration of 20 mg/L was added to the liquid medium immediately after the inoculation of the yeast. As indicated in Fig. 3.5,

Se was accumulated gradually with the increase of DCW and of the number of cells. The incorporated Se was of 5.16 µg/mL for 12 h and 6.91 µg/mL for 24 h. It can be concluded that a shorter yeast cultivation period is more efficient than a longer yeast cultivation period. The substrate consumption increased dramatically after 8 h fermentation, as the yeast growth cycle entered exponential phase. In this case, carbon sources and nitrogen source might be not enough for substrate consumption which become limiting factor after fermentation at 48 h, the result showed that the cell concentration were decrease. The final biomass reached 0.96 mg/mL and the Se was accumulated gradually with the increase of DCW (6.61 µg/mg DCW), the Se uptake level achieved 6.31µg/mL (Fig 3.5).

Moreover, *S. bayanus* showed the highest and stable to Se accumulation potential from 24 to 48 h. The Se was accumulated gradually with the increase of DCW (6.43×10^{-3} µg/mg DCW), the Se uptake level achieved 6.91×10^{-3} µg/mL and the biomass reached 1.08 mg/mL.

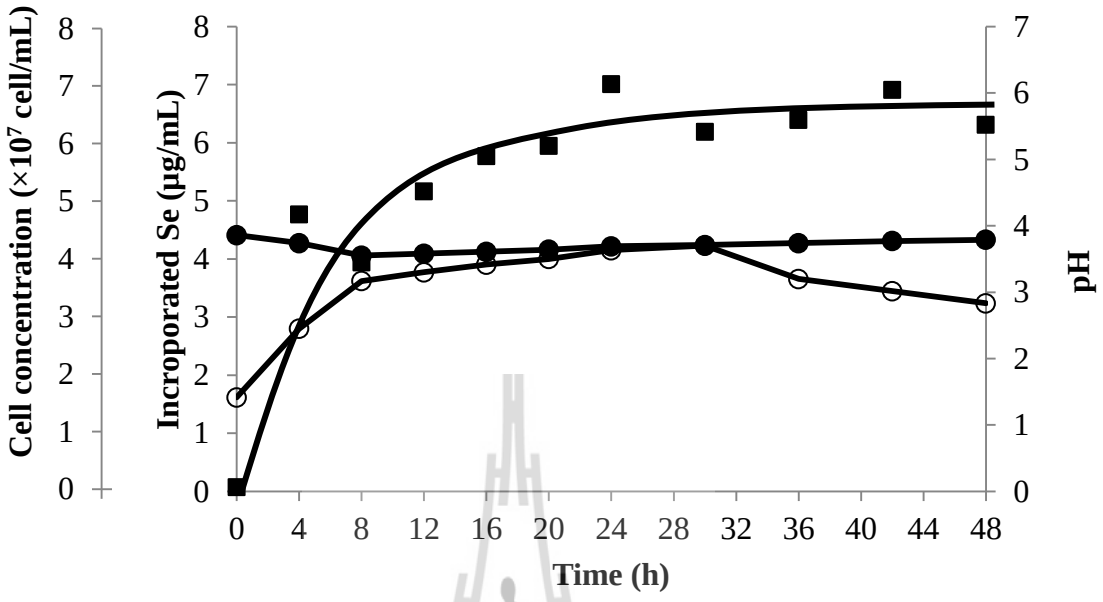

Figure 3.5 The effect of Na_2SeO_3 single addition on Se accumulation, pH in a 5 L fermentor at 48 h: total cell yeast (○), in corporate Se (■), pH (●)

3.5 Conclusions

Organically bound Se yeast was produced with high cell density fermentation. *S. bayanus* was tested and 0.96 mg/mL dry cell weight was obtained after 48 h cultivation in a 5 L fermentor. The optimal pattern of Na_2SeO_3 addition was 20 mg/L. To balance the Se incorporation and optimum growth of the yeast, the fermentation was used for conversion of organic Se by the single dose addition of Na_2SeO_3. At 48 h, the biomass reached 0.96 mg/mL. The Se was accumulated gradually with the increase of DCW (6.61 µg/mg DCW) and the Se uptake level achieved 6.31 µg/mL, which exhibited a potential for industrial application. Future work, Organic Se and inorganic Se will be tested efficiency of reproductive in boar.

3.6 References

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CHAPTER IV

EFFECT OF ORGANIC SELENIUM AND INORGANIC SELENIUMIN DIET OF SPERM QUALITY OF BOAR

4.1 Abstract

The objective of this study was to evaluate the short-term effect of dietary Se-yeast, yeast and Na_2SeO_3 on semen parameters in boar. A total of 24 boars were randomly assigned to ten treatment groups. The boars of diet 1 received a commercial feed, diet 2 received a commercial feed supplemented with yeast 0.60 mg in 1 kg of diet, diet 3, 4, 5 contained the following supplement, 0.15, 0.45 and 0.60 mg Na_2SeO_3/kg of diet, respectively. The diets 6, 7, 8 contained the following supplements, 0.15, 0.45, 0.60 mg of Se-yeast/kg of diet, respectively. Semen characteristics of sperm abnormal, sperm viability, sperm motility, volume, concentration and total sperm were recorded.

The result showed that short term dietary Se supplementation has effects on semen parameters. It could be concluded that the short-term supplementation of Na_2O_3Se and Se yeast in boar diet increase and maintain sperm motility, sperm volume higher than commercial feed ($P>0.05$), in contrast , the sperm abnormalities of boar were decreased, but did not have effect on sperm viabilities, sperm concentration and total sperms ($x10^6$/ejaculations) of boar ($P>0.05$). The supplementations of Na_2SeO_3 and Se yeast in boar not have effect on boar health because hematological and biochemical values in blood of boar were within the normal range of domestic pigs.

4.2 Introduction

Se is usually present at very low concentrations in the diet of farm animals. Regarding male reproduction, Se has been found in high concentrations in testis and epididymides of boars and is therefore likely important for the production and maturation of sperm (Marin-Guzman et al., 1997, 2000; Lasota et al., 2004).

For growing boars, it has been shown that higher levels of Se and vitamin E in the feed (0.5 mg Se and 60 mg vitamin E compared with 0.2 mg Se and 30 mg vitamin E/kg) improved semen quality (Kołodziej and Jacyno 2005). A positive effect of Se was found in relation to sexual activity of boars, their semen quality (motility, concentration, and sperm morphology) as well as to fertilization rates (Hansen and Deguchi, 1996; Kolodziej and Jacyno, 2004; Kołodziej and Jacyno, 2004). Another study reported that a basal diet for boars supplemented with Se (0.10 and 0.25 ppm Se) decreased libido and testis size compared with a basal diet poor in Se (0.005 ppm) (Henson et al., 1983). Glutathione peroxidase (GPx) activity has been found in sperm of boars (Lasota et al., 2004; Jelezarsky et al., 2008) and therefore a higher resistance to oxidative stress should be expected when Se is provided. Never the less, the way GPx acts on sperm protect ion remains unclear.

As a source of Se, the cited authors used inorganic compounds of this element (selenates and selenites). For pigs, Se is available both in the form of organic and inorganic compounds; however, the availability of organic compounds, as well as their biological efficiency are significantly higher than those of inorganic selenium compounds. Mahan and Parret (1996) found that the organic Se retention in pigs was by 85% higher than that of inorganic Se. Studies on boars have demonstrated that the organic Se is much more positive to reproduction of the males compared with an

inorganic form of this element (Mahan et al., 2002). The aim of this study was to compare reproductive performance of boars fed during their rearing on the diet supplemented with inorganic Se and organic Se.

4.3 Materials and methods

4.3.1 Animals and feeding

Twenty eight boars were included in this study. The age distribution was similar between 1.6 to 2.0 year of age; they were housed in individual pens within the same building and before the start of the trial. They received a commercial feed 2.5 kg/day, until the first day 1 of trial and then the boars were distributed into 10 groups, 3 boars each. During the test, the feeding of each group was differentiated only by concentration and chemical form of Na_2SeO_3, Se yeast and yeast. The boars of diet 1 received a commercial feed, diet 2 received a commercial feed supplemented with yeast 0.60 mg/kg of diet, diet 3, 4, 5 contained the following supplement, 0.15, 0.45 and 0.60 mg Na_2SeO_3/kg of diet, respectively. The diets 6, 7, 8 contained the following supplements, 0.15, 0.45, 0.60 mg Se-Yeast /kg diet, respectively. The boars were fed twice daily (09.00 am and 03.00 pm), they received each time 1.25 kg /day. The frequency of semen collection was one time/week. The animals were kept in individual pens throughout the test. Standard feeding was applied with permanent water supply.

4.3.2 Proximate composition analyses

The proximate analyses of diet were performed according to the standard methods of AOAC (1990) for dry matter, protein, total lipid, fiber and ash. Protein was determined by Kjeldahl method. Lipid content of samples was determined by petroleum ether extraction using a Soxtec System (Soxtec 2050; Auto Fat

Extraction System, Foss Tecator, Höganas, Sweden). Fiber content of sample was determined by using a Systems Fibertec (Fibertec 2010; Auto Fiber Analysis System, Foss Tecator, Höganas, Sweden). Samples used for ash content determination were incinerated in a muffle furnace at 600ºC for 3 h. Proximate composition of experimental diets was determined in triplicate using the same procedures.

4.3.3 Evaluation of the boars

The semen collections started and were continued until obtaining fully quality ejaculates, which were diagnosed. During the semen collection, sexual activity of the males was evaluated, measured as a number of leaps, time to effective phantom mounting, as well as the time of ejaculation.

4.3.4 Semen evaluation

Semen of the boars was collected according to standard procedures by means of the gloved hand technique (Shipl, 1999). Immediately after the collection and filtration of ejaculate, its following characteristics were determined: sperm abnormalities, sperm viabilities, sperm motilities, volume, concentration and total sperm, pH, temperature. In the seminal plasma, obtained by centrifugation of the liquid fraction of the semen. The seminal plasma had been stored at -20°C before the analyses took place.

- **Determination of semen volume, sperm concentration and motility**

Semen volumes were determined with a graduated cylinder. Sperm concentrations were estimated by Sperma Cue (Minitube, Germany). The percentage of motile sperm was estimated at 38.5°C using phase contrast microscopy at 40X. Sperms were fixed with eosin-nigrosin (1% eosin, 10% nigrosin) to examine viable

and acrosome morphology. One hundred sperms per sample were evaluated by phase contrast microscopy at 1,000X (Yi et al., 2008).

4.3.5 Hematological assays

Blood samples were collected aseptically from the jugular vein from all pigs on before and after finished treatment. Blood was collected in tubes containing EDTA for homological assay, Tubes without anticoagulant were used to collect serum for biochemical and cytokine measurements. Plasma and serum samples were collected after and stored at -20ºC until analyzed.

- **Hematology and Leukocytic values**

Red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), lymphocyte, monocyte, eosinophil, and basophil were determined using HMX Hematology Analyzer (coulter, Canada).

- **Blood biochemistry**

Cholesterol, triglyceride, High density lipoprotein (HDL), Low density lipoprotein (LDL), total protein, Albumin, Total bilirubin, Direct bililubin, aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), blood urea nitrogen (BUN), creatinine were determined using Automatic clinical chemistry analyzer (Biosystems A15, Canada).

4.3.6 Analysis of data

Analysis of variance (ANOVA) performed on all experimental data and means compared using the Duncan's New Multiple Rang Test with SPSS program version 13. The significance level was $p<0.05$ unless otherwise stated.

4.4 Results

4.4.1 Evaluate sperm of the ejaculation

Sperms were fixed with eosin-nigrosin (1% eosin, 10% nigrosin) to examine viable and acrosome morphology (Fig 4.1).

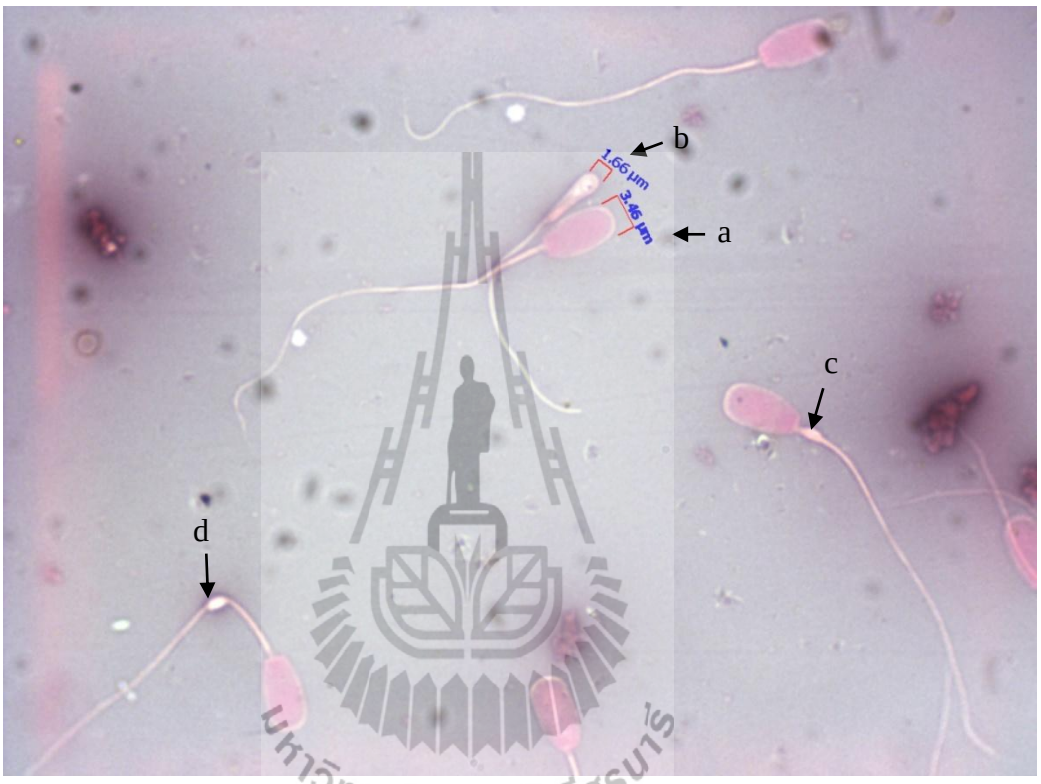

Figure 4.1 Sample images of boar sperm using a phase-contrast microscopy; normal sperm (a), malformed head (b), proximal protoplasmic droplet (c), bent tail (d)

4.4.2 The effect of Na_2O_3Se, Se-yeast and yeast on sperm

The sperm abnormal (%) of boar were shown in Fig. 4.2. The overall decreased in these abnormalities, for diet 7, the sperm abnormal (%) of boar on day 7, 14, 21, 28 were significantly lower than that of boar before treatment of 7 days (at day -7), ($P<0.05$), indicated that the supplementation of Na_2O_3Se and Se-yeast in boar diet decreased sperm abnormality of boar, not significant different between group

(P>0.05). Similar results were also mentioned in boar supplementation of Se and vitamin E can enhance boar's sperm quality by reducing abnormal morphology, resulting in higher fertilization rates (Marin-Guzman et al., 1997). The supplementations of Na_2SeO_3 and Se-yeast in boars' diet were decreased abnormalities of boar' sperm because Se is essential for the normal development of spermatozoa and is incorporated into the mitochondrial capsula protein. Se is also a component of GPx, an enzyme that protects cellular components against free radicals and is an antioxidant for cellular membrane lipids (Hansen and Deguchi, 1996).

For the sperm motilities of boar were shown in Fig. 4.3, the overall in motility of sperm higher than diet 1, indicated that the supplementation of Na_2O_3Se and Se-yeast, Se in boar diet increase and maintain sperm motility of boar (P>0.05). These result could only partially confirm the results of a previous study, Se has been proven to improve motility in the sperm from boars supplemented with dietary Na_2O_3Se from weaning to sexual maturity (Marin-Guzman et al., 1997).

Se in boar diets were increase and maintain sperm motility of boar because Se containing polypeptide found in rat sperm within a keratinous fraction mitochondrial capsule of the sperm (Calvin, 1978). This protein is localized in the mid piece portion of the sperm, is associated with a specific keratin within a fibrous fraction of the sperm tail (Brown and Burk, 1973; McConnell and Burton, 1981), and is necessary for the flagella integrity of the sperm. The protein has been reported to contain 3 selenocysteine residues (Karimpour et al., 1992). Se deficiency negatively affects the synthesis of the sperm mitochondrial capsule and may reduce sperm motility and impaired spermatogenesis. The sperm volume of boar was demonstrated in Fig. 4.4, the overall in volume of sperm higher than diet 1, indicated that the supplementation of Na_2O_3Se and Se-yeast in boar diet increase and maintain sperm

volumes of boar ($P>0.05$). Similar results were also in boar supplemented with Se, the volume of ejaculate was similar as age increase (Marin Guzman et al., 2009). Generally, research has shown that Se supplementation in boars increased Se concentrations in prostate gland, seminal vesicle, and bulbo urethral gland (Marin-Guzman et al., 1997; Segerson et al., 1981), the seminal vesicles produce the bulk of the ejaculate and fructose to nourish the sperm.

The prostate gland provides other nutrients and the bulbo rethral gland produce the jelly. Whereas, the sperm viabilities of boar were show in Fig. 4.5 The sperm viability of boar on diet 2 was significantly lower than that of boar on diet 1 (at day 21), ($P<0.05$), indicated that the supplementation of Na_2O_3Se and Se-yeast in boar diet did not have effect on sperm viabilities of boar, but the supplementation of yeast were decreased sperm viability of boar ($P<0.05$). However the sperm concentrations (Fig. 4.6) and total sperm (Fig 4.7) of boars were not significant between groups of diets These data any indicated that the supplementation of Na_2O_3Se, Se-yeast and yeast in boar diet did not have effect on sperm concentration and total sperm.

The previous reported that spermatozoal concentrations in ejaculated boar semen over a 16 week collection period were similar when boars were fed either a low-Se (0.06 mg/kg) or a Se-supplemented (0.5 mg.kg) diet (Marin-Guzman et al., 1997).

Table 4.1 Proximate composition (%) of eight experimental diets (mean ± SD, n=3)[1]

Ingredient (%)	Diet							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dry matter	92.43±0.19	92.49±0.19	92.65±0.03	92.94±0.26	92.47±0.11	92.70±0.35	92.44±0.19	92.60±0.15
Crude protein	17.41±0.10	17.04±0.53	16.85±0.51	17.70±0.09	17.52±0.10	17.45±0.46	17.53±0.08	17.50±0.06
Crude lipid	4.38±0.16	4.40±0.20	4.45±0.16	4.60±0.03	4.39±0.16	4.41±0.20	4.55±0.07	4.62±0.02
Crude fiber	5.88±0.37	6.34±0.68	6.42±0.16	6.16±0.15	5.86±0.30	6.74±0.15	6.29±0.12	6.55±0.04
Ash	0.07±0.00	0.07±0.01	0.08±0.00	0.08±0.01	0.07±0.00	0.07±0.01	0.08±0.01	0.07±0.00

[1]No significant differences (P>0.05) were observe amount treatment means

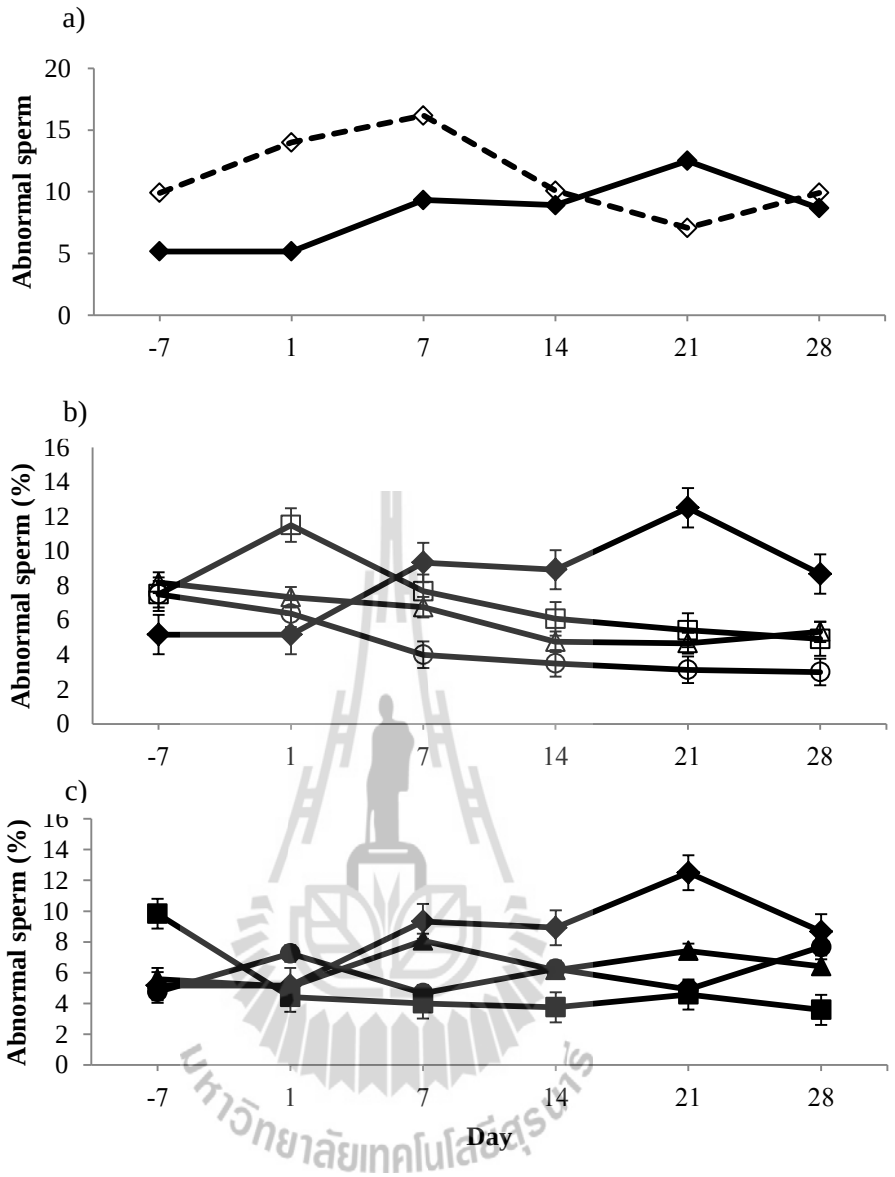

Figure 4.2 Effect of various dietary sperm in group of control, a); yeast and commercial, b); inorganic Se and Se-yeast c). Diet 1 (◆) received a commercial feed, diet 2 (◇) received a commercial feed supplemented with yeast (0.60 mg/kg of diet), diet 3, 4, 5 contained following supplement 0.15 (△), 0.45 (□), 0.60 (○) mg/kg of diet (Na_2O_3Se) respectively. The diets 6, 7, 8 contained following supplement contained the following supplement 0.15 (▲), 0.45 (■), 0.60 (●) mg/kg of diet) on the percentage of abnormal sperm for consecutive 28 days collection period in boar.

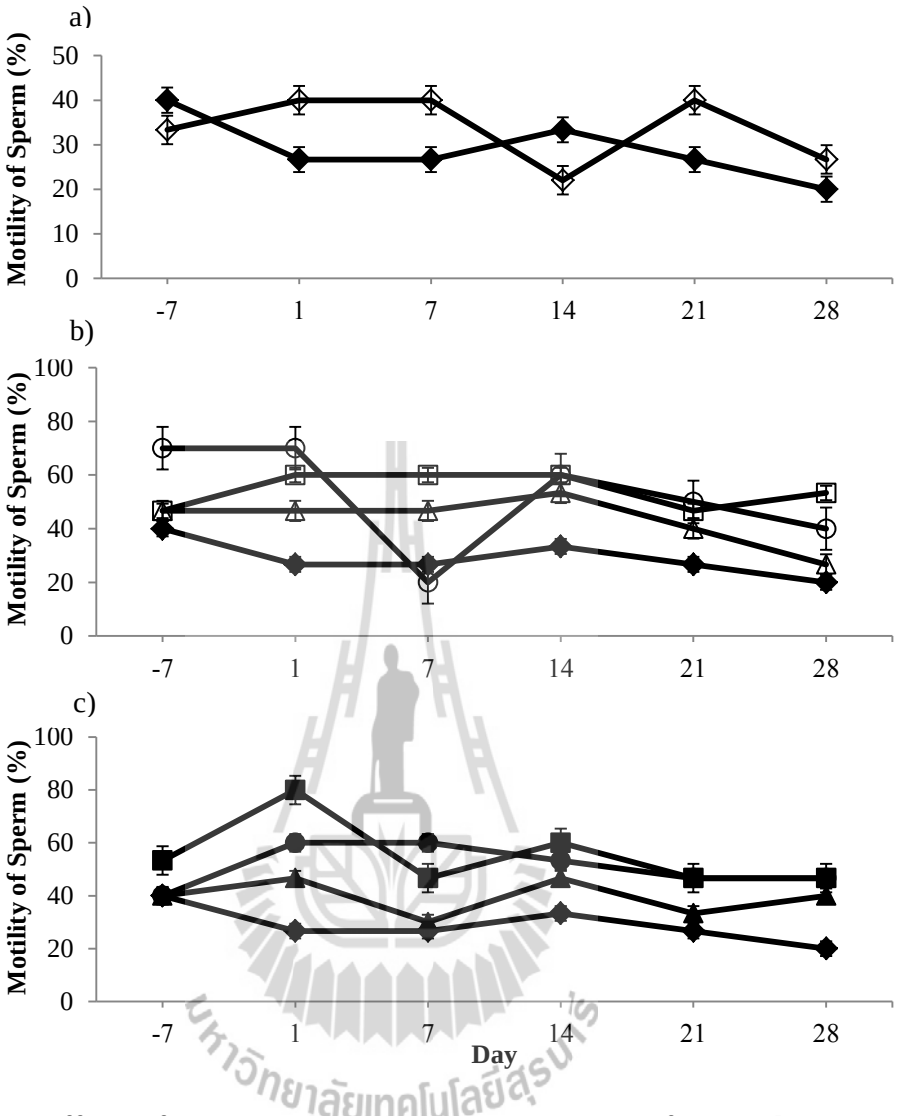

Figure 4.3 Effect of various dietary sperm in group of control, a); yeast and commercial, b); inorganic Se and Se-yeast c). Diet 1 (◆) received a commercial feed, diet 2 (◇) received a commercial feed supplemented with yeast (0.60 mg/kg of diet), diet 3, 4, 5 contained following supplement 0.15 (△), 0.45 (□), 0.60 (○) mg/kg of diet (Na_2O_3Se) respectively. The diets 6, 7, 8 contained following supplement contained the following supplement 0.15 (▲), 0.45 (■), 0.60 (●) mg/kg of diet) on the percentage motility of sperm for consecutive 28 day collection period in boar. The overall in motility of sperm higher than diet 1 (P>0.05).

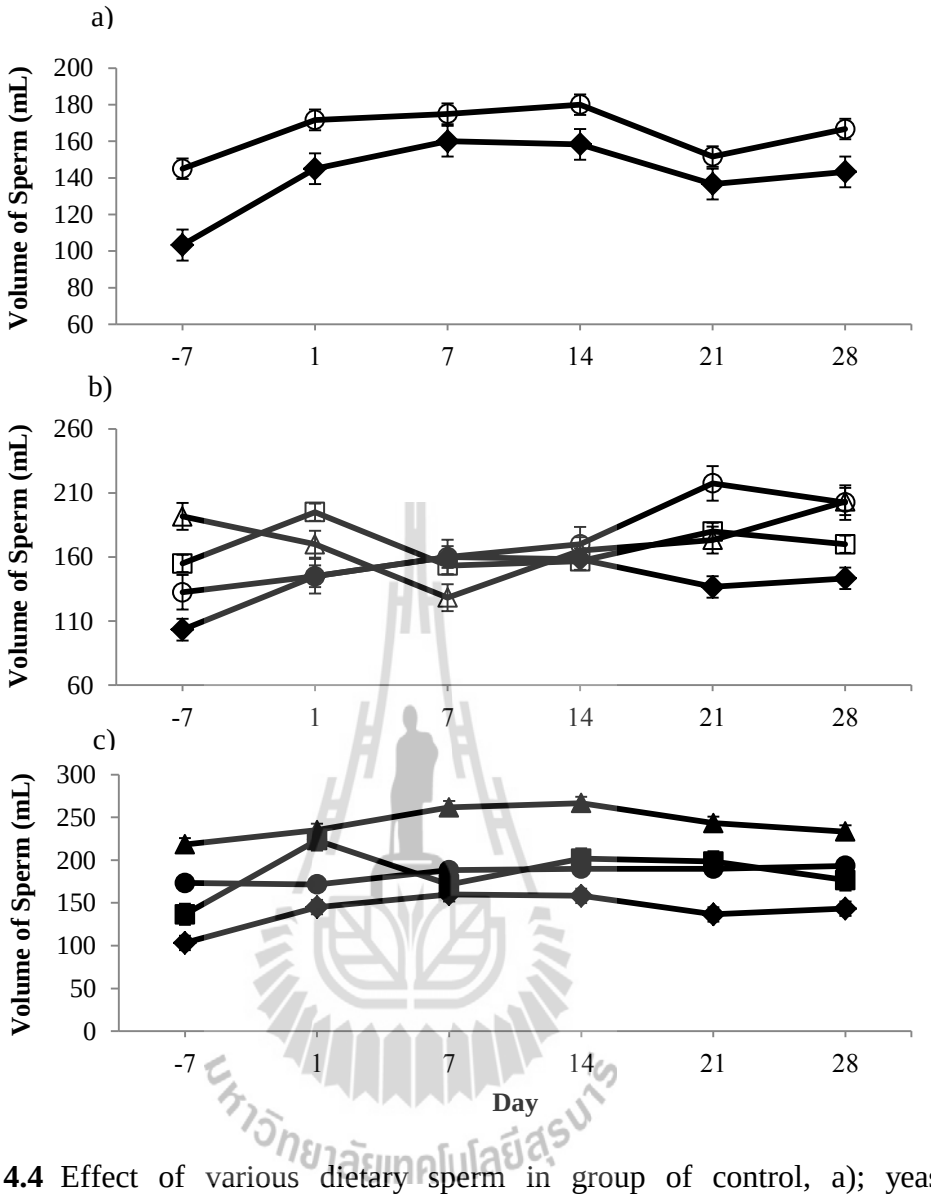

Figure 4.4 Effect of various dietary sperm in group of control, a); yeast and commercial, b); inorganic Se and Se-yeast c). Diet 1 (◆) received a commercial feed, diet 2 (◇) received a commercial feed supplemented with yeast (0.60 mg/kg of diet), diet 3, 4, 5 contained following supplement 0.15 (△), 0.45 (□), 0.60 (○) mg/kg of diet (Na_2O_3Se) respectively. The diets 6, 7, 8 contained following supplement contained the following supplement 0.15 (▲), 0.45 (■), 0.60 (●) mg/kg of diet) on the volume of sperm for consecutive 28 day collection period in boar. The overall in volume of sperm was significantly different higher (P>0.05) than diet 1

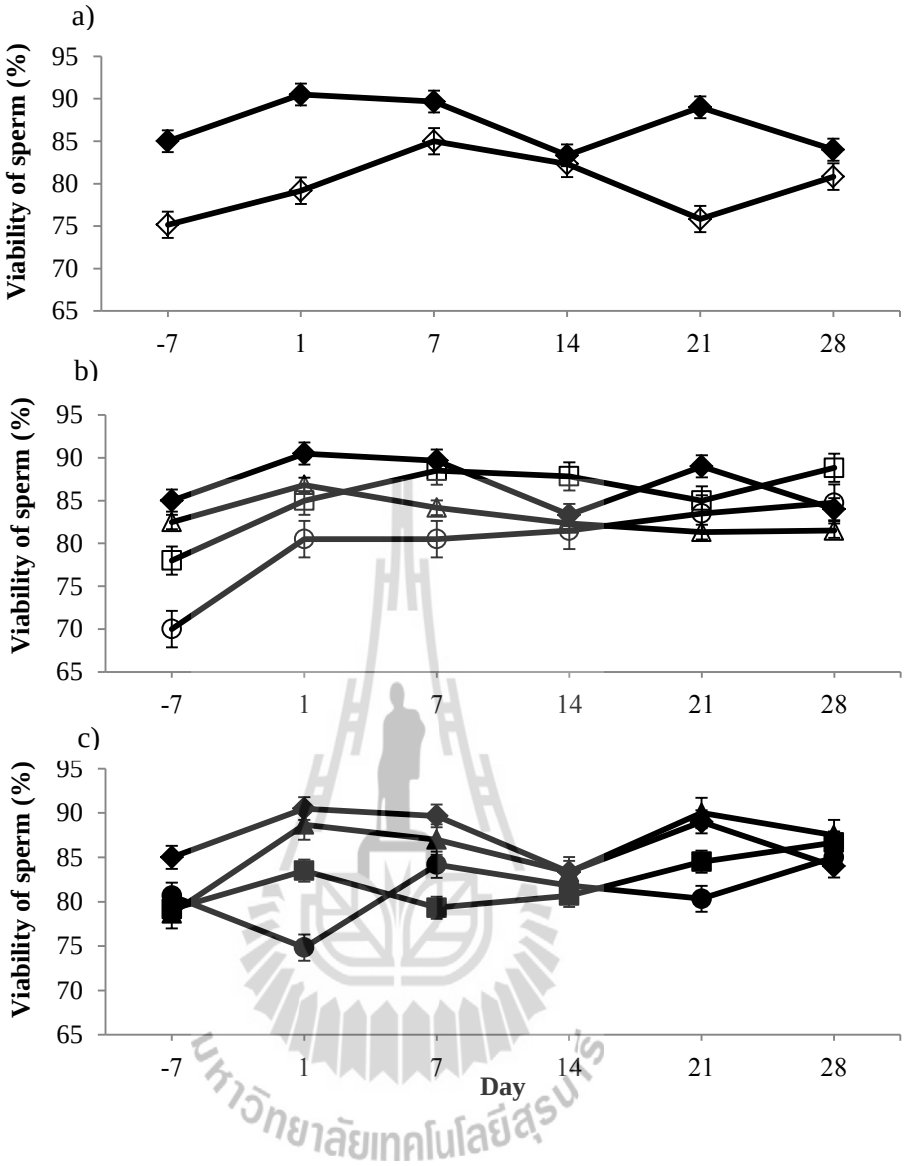

Figure 4.5 Effect of various dietary sperm in group of control, a); yeast and commercial, b); inorganic Se and Se-yeast c). Diet 1 (◆) received a commercial feed, diet 2 (◇) received a commercial feed supplemented with yeast (0.60 mg/kg of diet), diet 3, 4, 5 contained following supplement 0.15 (△), 0.45 (□), 0.60 (○) mg/kg of diet (Na_2O_3Se) respectively. The diets 6, 7, 8 contained following supplement contained the following supplement 0.15 (▲), 0.45 (■), 0.60 (●) mg/kg of diet) on the percentage viability of sperm for consecutive 28 day collection period in boar.

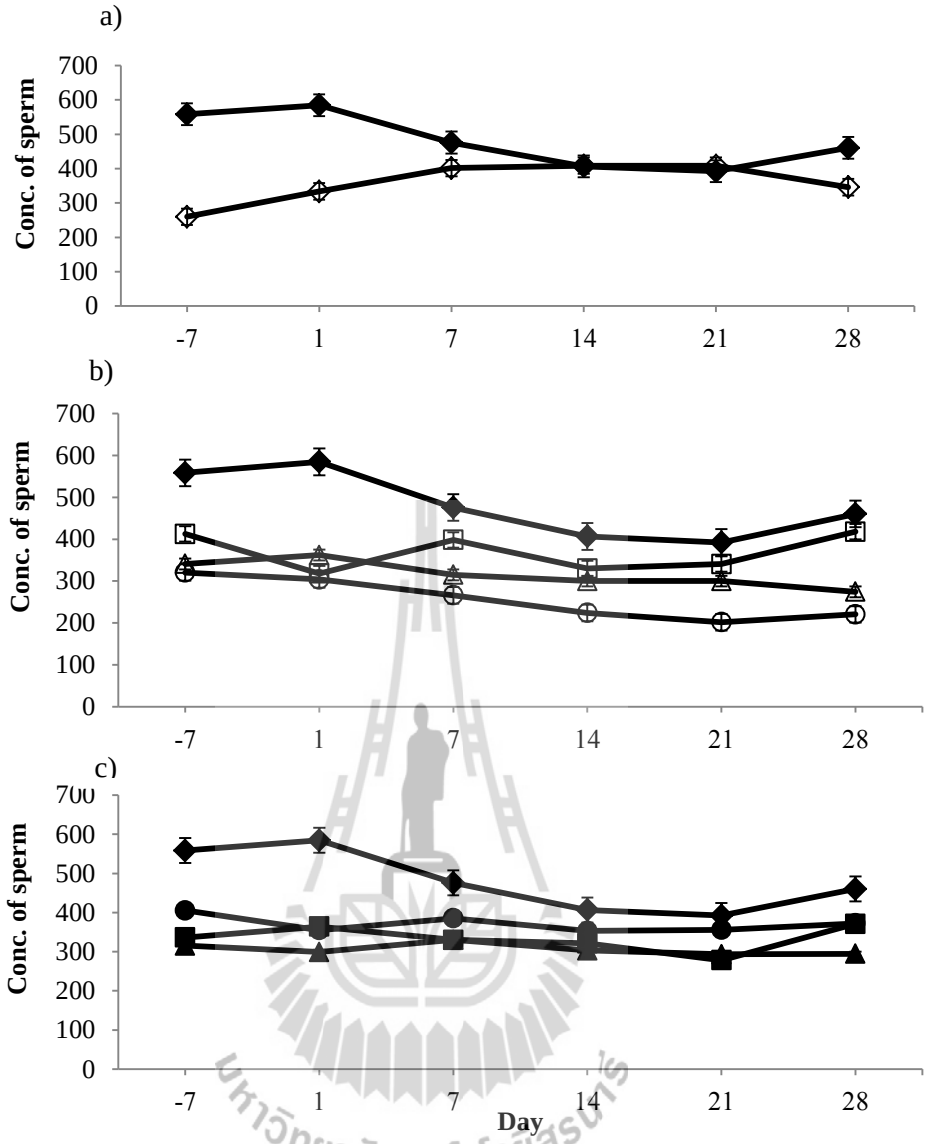

Figure 4.6 Effect of various dietary sperm in group of control, a); yeast and commercial, b); inorganic Se and Se-yeast c). Diet 1 (◆) received a commercial feed, diet 2 (◇) received a commercial feed supplemented with yeast (0.60 mg/kg of diet), diet 3, 4, 5 contained following supplement 0.15 (△), 0.45 (□), 0.60 (○) mg/kg of diet (Na_2O_3Se) respectively. The diets 6, 7, 8 contained following supplement contained the following supplement 0.15 (▲), 0.45 (■), 0.60 (●) mg/kg of diet) on the concentration of sperm ($x10^6$) for consecutive 28 day collection period in boar. The overall did not significantly different ($P>0.05$) between group of diet.

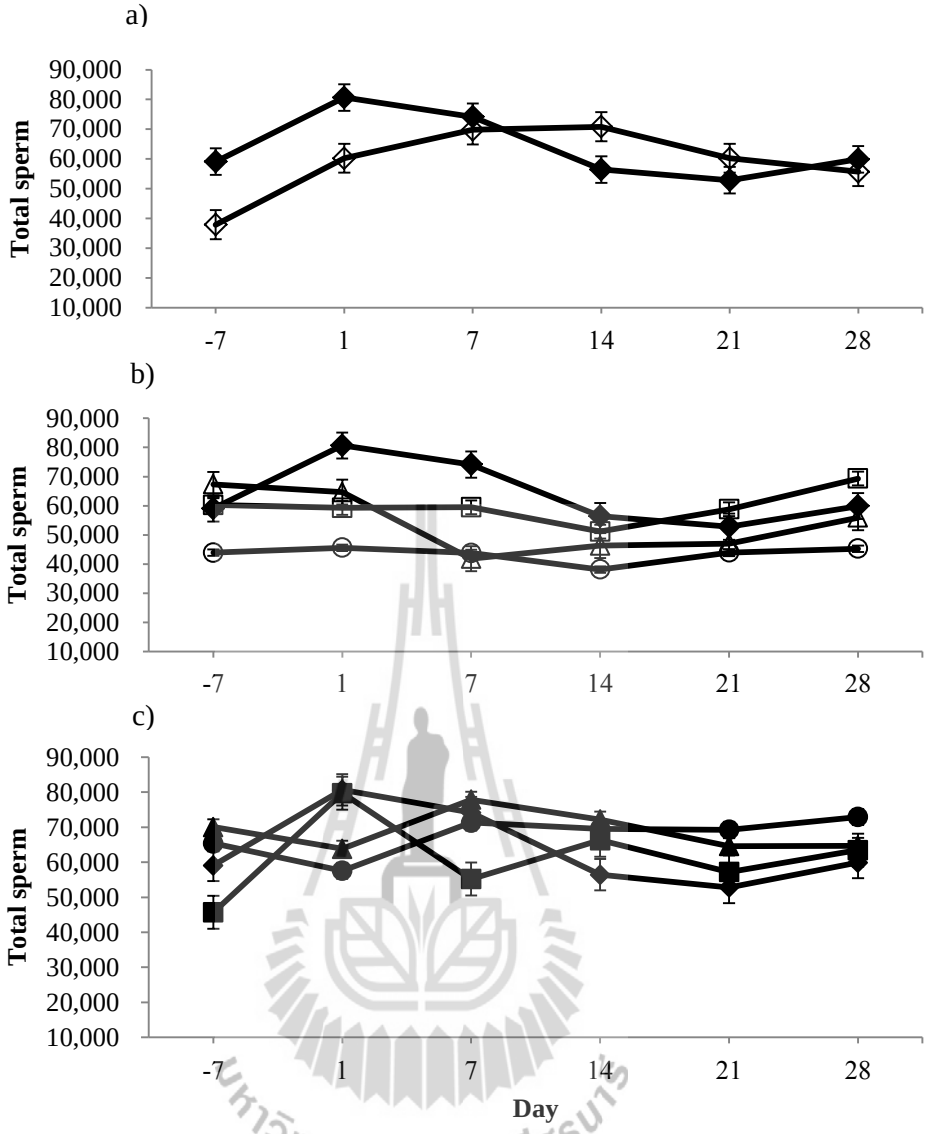

Figure 4.7 Effect of various dietary sperm in group of control, a); yeast and commercial, b); inorganic Se and Se-yeast c). Diet 1 (◆) received a commercial feed, diet 2 (◇) received a commercial feed supplemented with yeast (0.60 mg/kg of diet), diet 3, 4, 5 contained following supplement 0.15 (△), 0.45 (□), 0.60 (○) mg/kg of diet (Na_2O_3Se) respectively. The diets 6, 7, 8 contained following supplement contained the following supplement 0.15 (▲), 0.45 (■), 0.60 (●) mg/kg of diet) on the total of sperm ($x10^6$/ejaculation) for consecutive 28 day collection period in boar. The overall did not significant different ($P>0.05$) between group of diet.

4.4.3 Hematological and biochemical values blood of boar

Serum biochemical and leukocytic values compared favorably with those in domestic other population's data. Differences in some biochemical values were probably due to dietary differences and stress factors.

RBC, Hemoglobin, Hematocrit, MCV, MCH and MCHC levels were within the normal range of values in domestic pigs. For leukocytic analyses, WBC , Lymphocyte, Monocyte, Eosinophil, Basophil, levels were within the normal range of values in domestic pigs.

- **Blood biochemistry for lipid profile, liver and kidney function**

Cholesterol, triglyceride, HDL, LDL, Cholesterol, triglyceride levels were within the normal range of values in domestic pigs. The HDL of boar on Diets 1 levels were less than the normal range of values in domestic pig whereas Diets 5, 7 were higher than the normal range of values in domestic pig but Diets 2, 3, 4, 6, 8 levels were within the normal range of values in domestic pig. The LDL of boar on Diets 1 was higher than the normal range of values in domestic pig. For liver and kidney function test the total protein, Albumin, total bilirubin, direct bilirubin, SGOT, SGPT and BUN levels were within the normal range of values in domestic pigs. Whereas, creatinine of boar on Diets 1, 2, 5 were slightly higher than the normal range of values in domestic pig 1.0-2.7 mg/dl (hear, www. 2012).

Previous research has shown a range of mean values of HDL in boar from 39-45 mg/dL (Martin Rodriguez et al., 2002). In this study, HDL values were described to be as low as 27 mg/dL and as high 53 mg/dL. Although HDL values from pigs in this study were within a range of normal values for healthy pigs, but pigs in

diet 1 had a decrease HDL by 27 mg/dL compared to pigs in domestic pig (39-45 mg/dL), this decrease may indicate that pigs in diet 1 shower hepatitis and cirrhosis because liver synthesizes HDL, which transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by direct and indirect pathways (Wikipedia, www. 2012).

Pigs in diet 5 and 7 were much higher than the normal range of values in domestic pig, this indicated that the pigs showed high HDL levels might correlate with better cardiovascular health because of HDL function for removing cholesterol from within artery atheroma and transport it back to the liver for excretion or re-utilization, which is the main reason why the cholesterol carried within HDL particles (HDL-C) is sometimes called "good cholesterol". Those with higher levels of HDL-C seem to have fewer problems with cardiovascular diseases, while those with low HDL-C cholesterol levels have increased rates for heart disease. While higher HDL levels are correlated with cardiovascular health. In other words, while high HDL levels might correlate with better cardiovascular health. Diet 2, 3, 4, 6 and 8 showed HDL within a range of normal values for domestic pigs. Therefore, inorganic Se, organic Se can increase and maintain HDL in blood biochemical of boar.

Boar LDL number in previous research has shown a range of mean values from 17-25 mg/dl (Martin Rodriguez et al., 2002). In these studies, LDL values were described to be as low as 20 mg/dl and as high 35 mg/dl. Although LDL numbers from pigs in this study were within a range of normal values for healthy pigs, pigs in Diet 1 had an increase LDL by 35 mg/dl compared to pigs in domestic pig (17-25 mg/dl). This increase indicated that pigs in diet 1 were showing abnormal kidney and then stimulated liver to synthesize LDL higher than the normal range of LDL. For

the cardiovascular disease, higher levels of LDL particles promote health problems and cardiovascular disease. LDL is often informally called the bad cholesterol particles (Wikipedia, www. 2012). Diet 2, 3, 4, 5, 6, 7 and 8 were shown LDL within a range of normal values for domestic pigs. Therefore, inorganic Se, organic Se can decrease LDL in blood biochemical of boar.

4.4.4 Blood biochemistry for kidney function test

Boar creatinine value in previous research has shown a range of mean values from 1.0 to 2.7 mg/dL (Hear, www. 2012). In these studies, creatinine values were described to be as low as 2.47 mg/dl and as high at 3.00 mg/dL. Although creatinine numbers from pigs in this study were within a range of normal values for healthy pigs, pigs in diet 1, 2 and 5 had an increase creatinine by 3.00, 2.77 and 2.79 mg/dl, respectively, compared to pigs in domestic pig (1.0-2.7 mg/dl), Creatinine is chiefly filtered out of the blood by the kidneys. If the filtering of the kidney is deficient, creatinine blood levels rise (Wikipedia, www. 2012), creatinine may increase to very high levels following kidney damage (Hear, www. 2012). Diet 3, 4, 6, 7 and 8 were shown creatinine within a range of normal values for domestic pigs. Therefore, inorganic Se, organic Se can decrease creatinine in blood biochemical of boar.

Bioavailability of Se depends on the species of animal, the source, level and on which chemical from of the element is present in the diet. In monogastric animal, organic Se is more effectively retained than inorganic Se that is commonly used in animal diets. Se yeast deposited in the muscular tissue better than Na_2O_3Se (Mahan and Parrett, 1996). For long term of supplementation of organic Se compounds is better and safe than that of inorganic Se. (Wikipedia, www. 2012).

The supplementation of Na_2SeO_3 and Se-yeast in boar have not effect on boar health, because hematological and biochemical values in blood of boar levels were within the normal range of values in domestic pigs.

Table 4.2 Hematology values of boar fed experimental diets (mean ± SD, n=3)[1].

Day 28	RBC (10^6/µL)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)
1	7.26±0.22	14.00±1.00	40.00±2.00	55.03±2.20	19.23±1.07	34.90±0.52
2	6.71±0.76	14.00±0.00	39.00±0.00	57.85±6.86	20.60±2.26	35.60±0.28
3	6.61±1.31	13.33±1.53	37.67±5.69	57.53±3.61	20.37±1.40	35.37±0.47
4	6.32±0.60	13.33±1.53	36.67±3.79	57.57±1.67	20.70±0.52	36.00±0.26
5	5.51±1.32	11.50±2.12	37.00±0.00	57.25±0.64	20.75±0.49	36.20±0.00
6	6.76±0.18	13.50±0.71	38.00±0.00	55.95±0.92	20.25±0.35	36.20±0.42
7	6.52±0.32	13.00±0.00	35.50±0.71	54.85±1.63	19.80±1.13	36.15±0.35
8	6.14±0.78	13.33±1.53	37.33±2.53	61.07±3.76	21.90±1.21	35.80±1.06

The normal range of values in domestic pigs; 5.0-8.0 RBC, 10.0-16.9 Hemoglobin, 35.6-62.10 Hematocrit, 54.0-73.0 MCV, 18.8-25.5 MCH, 32.2-36.5 MCHC

[1]No significant differences (P>0.05) were observe amount treatment mean

Table 4.3 Leukocytic values of boar fed experimental diets (mean ± SD, n=3)[1].

Day 28	WBC	Lymphocyte	Monocyte	Eosinophil	Basophil
	(10^3/µL)	(%)	(%)	(%)	(%)
1	14.95±0.07	52.33±16.65	5.00±3.00	7.67±5.86	1.67±2.89
2	15.35±0.21	55.00±4.24	3.50±0.71	7.50±4.95	0.00±0.00
3	16.00±1.25	56.33±10.41	5.00±2.65	6.67±2.52	0.67±0.58
4	14.13±0.67	57.00±9.17	5.00±1.00	4.67±2.52	0.33±0.58
5	15.80±0.00	69.00±26.87	5.00±5.66	6.00±4.24	0.50±0.71
6	15.75±4.88	59.50±7.78	4.00±0.00	6.50±0.71	1.50±0.71
7	10.25±4.31	57.00±9.90	3.00±1.41	3.50±0.71	1.00±1.41
8	13.75±3.45	57.67±6.66	4.33±3.60	1.33±0.58	0.67±0.58

The normal range of values in domestic pigs; 4.7-18.6 WBC, 19.2-72.0 Lymphocyte, 0.0-8.0 Monocyte, 1.0-11.0 Eosinophil, 0.0-2.0 Basophil

[1]No significant differences (P>0.05) were observe amount treatment mean

Table 4.4 Biochemical of boar fed experimental diets (mean ± SD, n=3)[1].

Day 28	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	69.33±16.56	38.33±17.04	27.00±7.02	35.00±7.55
2	63.00±24.04	34.50±21.92	41.00±0.00	23.50±10.61
3	67.00±13.23	21.33±9.50	44.00±7.00	29.00±0.00
4	59.50±10.79	18.33±5.00	44.67±2.00	22.00±5.86
5	70.00±11.31	30.00±13.01	53.00±0.00	22.50±0.71
6	59.00±26.21	30.33±12.50	41.67±13.20	24.33±5.51
7	71.00±25.46	34.00±2.83	49.00±6.36	23.50±4.95
8	55.00±1.15	39.00±21.93	45.00±7.07	20.00±4.36

The normal range of values in domestic pigs; 50.0-140.0 Cholesterol, 14.0-70 Triglyceride, 39-45 HDL, 17-25 LDL

[1]No significant differences (P>0.05) were observe amount treatment mean

Table 4.5 Biochemical of boar fed experimental diets (mean ± SD, n=3)[1].

Day 28	Total protein (g/dL)	Albumin (g/dL)	Total bililubin (mg/dl)	Direct bililubin (mg/dl)	SGOT (U/L)	SGPT (U/L)
1	8.53±0.68	3.23±0.57	1.03±0.32	0.37±0.12	33.00±1.41	50.50±16.26
2	7.55±0.64	3.50±0.14	0.70±0.99	0.10±0.14	51.00±32.53	71.50±31.82
3	8.37±0.38	3.37±0.64	0.57±0.31	0.10±0.00	32.00±3.61	34.00±7.00
4	7.73±0.72	3.27±0.60	0.50±0.44	0.07±0.12	27.50±2.12	26.50±4.95
5	8.40±0.14	3.45±0.07	1.05±0.78	0.45±0.35	38.50±13.44	32.50±7.78
6	7.70±0.20	3.27±0.50	0.87±0.40	0.13±0.15	47.00±7.07	43.00±5.66
7	7.55±1.20	3.60±0.71	1.15±0.35	0.20±0.00	31.50±12.02	31.50±13.44
8	8.23±0.93	3.50±0.46	0.80±0.61	0.20±0.10	27.50±7.78	55.50±0.71

The normal range of values in domestic pigs; 6.7-13.8 Total protein, 2.7-3.9 Albumin, 0.4-1.7 Total bililubin, 0.0-0.5 Direct bililubin, 15.0-135.0 SGOT, 13-145 SGPT

[1]No significant differences (P>0.05) were observe amount treatment mean

Table 4.6 Biochemical of boar fed experimental diets (mean ± SD, n=3)[1].

Day 28	BUN (mg/dl)	Creatinine (mg/dl)
1	8.80±1.39	3.00±0.25
2	10.55±2.47	2.77±0.00
3	10.60±2.26	2.47±0.34
4	9.03±1.72	2.59±0.33
5	10.85±0.35	2.79±0.00
6	11.10±1.23	2.65±0.09
7	9.40±0.28	2.51±0.00
8	10.07±1.23	2.56±0.09

The normal range of values in domestic pigs; 8.0-24.0 BUN, 1.0-2.7 creatinine

[1]No significant differences (P>0.05) were observe amount treatment mean

4.5 Conclusions

The supplementation of Se-yeast and Na_2SeO_3 in boar diets can increase and maintain sperm motility and volume, which were higher than those of commercial feed (P>0.05). Moreover, the sperm abnormalities of boar were decreased whereas sperm viabilities, sperm concentration and total number of sperms were not significantly different (P>0.05) in all treatments. Finally, the supplementations of Se-yeast and Na_2SeO_3 in boar diets did not have effect on hematological and biochemical values in boar's blood.

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CHAPTER V

SUMMARY

Saccharomyces bayanus showed the highest to Se (10 mg/L Na_2SeO_3) accumulation potential. Increase of Se concentration from 10 to 60 mg/L diminished specific growth rate from 0.089 to 0.017 h^{-1} and the yeast growth was inhibited. *S. bayanus* showed the highest to Se (20 mg/L Na_2SeO_3) accumulation potential at 24-48 h and then the scale up experiments were carried out in a 5 L fermentor. Na_2SeO_3 with final concentration of 20 mg/L were added to the liquid medium immediately after the inoculation of the yeast.

Se was accumulated gradually with the increase of DCW and of the number of cells. The final biomass reached 0.96 mg/mL and The Se was accumulated gradually with the increase of DCW (6.61 µg/mg DCW), the Se uptake level achieved 6.31 µg/mL. However, *S. bayanus* showed the highest to Se accumulation potential at 24-42 h. The Se was accumulated gradually with the increase of DCW (6.43 µg/mg DCW), the Se uptake level achieved 6.91 µg/mL and the biomass reached 1.08 mg/mL. Se-yeast from this experiment, inorganic Se and yeast will be tested efficiency of reproductive in boar. Twenty four boars were included in this study.

The age distribution was similar namely 1.6 to 2.0 year of age, they were housed in individual pens within the same building and before the start of the trial, they received a commercial feed 2.5 kg/day, until the first day of trial, then the boars were distributed into 10 groups, 3 boars each. The boars of diet 1 received a

commercial feed, diet 2 received a commercial feed supplemented with yeast 0.60 mg/kg of diet, diet 3, 4, 5 contained the following supplement, 0.15, 0.45 and 0.60 mg Na_2SeO_3/kg of diet, respectively. The diets 6, 7, 8 contained the following supplements, 0.15, 0.45, 0.60 mg Se-Yeast /kg diet, respectively. The frequency of semen collection was one time/week. The diets of boar were proximate analyses for dry matter, protein, total lipid, fiber and ash, did not significant differences between diet groups ($P>0.05$).

The result showed that the supplementation of Na_2SeO_3 and Se-yeast in boar diets can increase and maintain sperm motility and volume, which were higher than those of commercial feed ($P>0.05$). Moreover, the sperm abnormalities of boar were decreased whereas sperm viabilities, sperm concentration and total number of sperms were not significantly different ($P>0.05$) in all treatments.

For boar health the supplementations of Na_2SeO_3 and Se-yeast in boar diets did not have effect on hematological and biochemical values in boar's blood and the values in blood of boar levels were within the normal range of values in domestic pigs and not significantly different between group ($P>0.05$).

APPENDICE

Table A. 1 Sperm abnormal of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	5.17 ± 5.54	5.17 ± 2.67 ^{b}	9.33 ± 6.57 ^{ab}	8.92 ± 9.05	12.5 ± 9.54	8.67 ± 7.23
2	9.92 ± 2.57	14.00 ± 8.67 ^{a}	16.17 ± 10.56 ^{a}	10.08 ± 7.47	7.08 ± 6.21	9.92 ± 8.99
3	8.17 ± 3.50	7.33 ± 2.63 ^{ab}	6.75 ± 3.47 ^{ab}	4.75 ± 3.85	4.67 ± 3.00	5.33 ± 6.68
4	7.50 ± 3.44 ^{AB}	11.5 ± 1.95 $^{ab, A}$	7.67 ± 2.47 $^{AB, ab}$	6.08 ± 1.88 ^{AB}	5.42 ± 2.38 ^{B}	4.92 ± 5.03 ^{B}
5	7.50 ± 1.41 ^{A}	6.38 ± 2.30 $^{ab, AB}$	4.00 ± 1.06 $^{b, B}$	3.50 ± 0.71 ^{B}	3.13 ± 0.18 ^{B}	3.00 ± 1.41 ^{B}
6	5.58 ± 7.07	5.08 ± 1.84 ^{b}	8.08 ± 2.32 ^{ab}	6.17 ± 2.31	7.42 ± 0.63	6.42 ± 7.89
7	9.83 ± 2.04 ^{A}	4.42 ± 2.27 ^{b,B}	4.00 ± 1.73 $^{b, B}$	3.75 ± 1.75 ^{B}	4.58 ± 2.32 ^{B}	2.88 ± 0.18 ^{B}
8	4.75 ± 1.64	7.25 ± 1.56 ^{ab}	4.67 ± 2.43 ^{b}	6.25 ± 4.79	4.92 ± 2.01	7.67 ± 8.14

^{ab}Means with different superscript in each column differed significantly from each other (P<0.05)

^{AB} Means with different superscript in each low differed significantly from each other (P<0.05)

Table A. 2 Sperm viability of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (Day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	85.00 ± 3.50 [a]	90.50 ± 1.00	89.67 ± 1.04	83.33 ± 8.10	89.00 ± 1.80 [ab]	84.00 ± 4.27
2	75.17 ± 9.93 [ab]	79.17 ± 6.83	85.00 ± 5.07	82.33 ± 4.37	75.83 ± 1.26 [d]	80.83 ± 7.01
3	82.50 ± 4.44 [ab]	86.83 ± 4.07	84.17 ± 2.57	82.33 ± 3.79	81.33 ± 5.39 [bcd]	81.50 ± 5.57
4	78.00 ± 5.27 [ab]	85.00 ± 10.58	88.50 ± 5.77	87.83 ± 9.12	85.00 ± 4.44 [abc]	88.83 ± 3.55
5	70.00 ± 10.61 [ab]	80.50 ± 20.51	80.50 ± 2.83	81.50 ± 7.07	83.50 ± 4.24 [abcd]	84.75 ± 9.55
6	78.68 ± 14.38 [ab]	88.68 ± 4.63	87.00 ± 4.00	83.33 ± 3.33	90.00 ± 2.00 [a]	87.50 ± 4.27
7	79.17 ± 6.35 [ab]	83.50 ± 8.79	79.33 ± 13.25	80.67 ± 4.01	84.50 ± 8.26 [abc]	86.67 ± 0.76
8	80.67 ± 3.33 [ab,AB]	74.83 ± 7.65 [B]	84.17 ± 5.01 [A]	81.83 ± 5.01 [AB]	80.33 ± 1.53 [cd,AB]	85.00 ± 3.12 [A]

[ab]Means with different superscript in each column differed significantly from each other (P<0.05)

[AB] Means with different superscript in each low differed significantly from each other (P<0.05)

Table A. 3 Sperm motility of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (Day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	40.00 ± 20.00	26.67 ± 11.55 [b]	26.67 ± 23.09	33.33 ± 11.55 [bc]	26.67 ± 11.55	20.00 ± 0.00
2	33.33 ± 30.55	40.00 ± 34.64 [ab]	40.00 ± 20.00	22.00 ± 33.05 [c]	40.00 ± 34.64	26.67 ± 23.09
3	46.67 ± 23.09	46.67 ± 11.55 [ab]	46.67 ± 11.55	53.33 ± 11.55 [ab]	40.00 ± 20.00	30.00 + 14.14
4	46.67 ± 41.63	60.00 ± 20.00 [ab]	60.00 ± 20.00	60.00 ± 0.00 [ab]	46.67 ± 11.55	53.33 ± 11.55
5	70.00 ± 14.14	70.00 ± 14.14[ab]	40.00 ± 28.28	60.00 ± 0.00 [ab]	50.00 ± 14.14	40.00 ± 0.00
6	40.00 ± 34.64	46.67 ± 30.55 [ab]	30.00 ± 14.14	46.67 ± 11.55 [abc]	33.33 ± 23.09	40.00 ± 20.00
7	53.33 ± 11.55	80.00 ± 0.00 [a]	46.67 ± 30.55	60.00 ± 30.55 [ab]	46.67 ± 11.55	46.67 ± 23.09
8	46.67 ± 23.09	46.67 ± 11.55 [ab]	46.67 ± 11.55	53.33 ± 11.55 [ab]	40.00 ± 20.00	30.00 + 14.14

[ab]Means with different superscript in each column differed significantly from each other (P<0.05)

[AB] Means with different superscript in each low differed significantly from each other (P<0.05)

Table A. 4 Sperm volume of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (Day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	103.33 ± 40.10 ^{b}	145.00 ± 65.00	160.00 ± 52.20 ^{ab}	158.33 ± 60.28	136.67 ± 40.41	143.33 ± 46.46
2	145.00 ± 5.00 ^{ab}	171.67 ± 92.51	175.00 ± 32.79 ^{ab}	180.00 ± 56.79	151.67 ± 33.29	166.67 ± 48.05
3	191.67 ± 37.53 ^{ab}	170.00 ± 50.00	128.33 ± 65.06 ^{B}	165.00 ± 49.24	173.33 ± 54.85	203.33 ± 36.17
4	155.00 ± 80.47 ^{ab}	195.00 ± 121.35	153.33 ± 67.52 ^{ab}	156.67 ± 61.10	180.00 ± 92.60	170.00 ± 65.57
5	132.50 ± 24.75 ^{ab}	145.00 ± 28.28	160.00 ± 28.28 ^{ab}	170.00 ± 77.78	217.50 ± 74.25	202.50 ± 60.10
6	218.33 ± 17.56 ^{a}	235.00 ± 58.95	261.67 ± 59.65 ^{a}	266.67 ± 67.52	243.33 ± 43.68	233.33 ± 76.54
7	136.67 ± 31.75 ^{ab,B}	223.33 ± 71.12 ^{A}	171.67 ± 27.54 ^{ab,AB}	201.67 ± 35.12 ^{AB}	198.33 ± 45.37 ^{AB}	176.67 ± 25.17 ^{AB}
8	173.33 ± 70.95 ^{ab}	171.67 ± 32.53	188.33 ± 45.37 ^{ab}	190.00 ± 25.00	190.00 ± 26.46	193.33 ± 14.43

^{ab}Means with different superscript in each column differed significantly from each other (P<0.05)

^{AB} Means with different superscript in each low differed significantly from each other (P<0.05)

Table A. 5 Sperm concentration of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (Day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	558.33 ± 87.84 [a]	584.67 ± 96.09 [a]	475.67 ± 79.48	406.67 ± 200.54	392.33 ± 195.68	460.33 ± 245.01
2	259.67 ± 107.85 [b]	333.67 ± 213.45 [b]	401.33 ± 78.36	408.67 ± 96.76	408.67 ± 90.74	346.00 ± 68.83
3	341.00 ± 99.96 [ab]	362.00 ± 101.98 [ab]	315.00 ± 93.64	300.00 ± 107.92	300.00 ± 147.00	274.33 ± 102.01
4	412.33 ± 83.44 [ab]	318.00 ± 45.57 [b]	398.33 ± 38.21	330.00 ± 15.87	340.67 ± 46.48	418.00 ± 112.90
5	320.50 ± 118.09 [ab]	304.00 ± 107.48 [b]	266.00 ± 86.27	224.00 ± 1.41	202.00 ± 1.41	220.50 ± 20.51
6	315.33 ± 138.91 [ab]	299.33 ± 165.46 [b]	330.33 ± 229.84	303.33 ± 195.05	293.33 ± 232.56	294.67 ± 173.81
7	336.33 ± 76.66 [ab]	364.00 ± 42.30 [ab]	330.33 ± 106.37	321.33 ± 132.67	278.33 ± 104.08	371.00 ± 135.01
8	405.33 ± 196.61 [ab]	355.33 ± 158.11 [ab]	385.33 ± 132.67	353.00 ± 153.73	356.00 ± 117.02	372.33 ± 152.64

[ab]Means with different superscript in each column differed significantly from each other (P<0.05)

[AB] Means with different superscript in each low differed significantly from each other (P<0.05)

Table A. 6 Total sperm of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (Day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	59,071.67 ± 26,998.83	80,660.00 ± 24,813.71	74,133.33 ± 20,497.09	56,433.33 ± 4,002.28	52,833.33 ± 33,407.55	59,898.33 ± 23,053.26
2	37,878.33 ± 16,796.26	60,225.00 ± 43,064.77	69,781.67 ± 15,331.50	70,811.67 ± 18,420.34	60,235.00 ± 5,686.70	55,713.33 ± 9,022.98
3	67,330.00 ± 31,401.02	64,656.67 ± 33,090.25	41,831.67 ± 30,562.37	46,328.33 ± 9,764.87	46,995.00 ± 15,878.40	55,923.33 ± 21,510.58
4	60,251.67 ± 24,220.43	59,281.67 ± 29,491.09	59,466.67 ± 22,026.12	51,140.00 ± 18,533.33	58,785.00 ± 23,457.07	69,320.00 ± 24,186.51
5	43,927.50 ± 23,578.48	45,600.00 ± 24,183.05	43,780.00 ± 21,326.34	38,135.00 ± 17,663.53	43,987.50 ± 15,305.33	45,267.50 ± 17,405.43
6	70,026.67 ± 33,151.29	63,845.00 ± 25,488.79	77,866.67 ± 43,457.81	72,178.33 ± 30,682.49	64,605.00 ± 40,194.72	64,710.00 ± 43,334.34
7	45,678.33 ± 15,178.76	79,716.67 ± 18,041.87	55,195.00 ± 13,561.73	66,306.67 ± 31,377.51	57,206.67 ± 27,652.92	63,470.00 ± 17,124.89
8	65,380.00 ± 26,642.17	57,570.00 ± 16,184.40	71,305.00 ± 27,259.55	69,536.67 ± 36,798.90	69,293.33 ± 30,188.37	72,898.33 ± 33,020.13

[ab]Means with different superscript in each column differed significantly from each other (P<0.05)

[AB] Means with different superscript in each low differed significantly from each other (P<0.05)

Table A. 7 Sperm temperature of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (Day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	32.83 ± 1.62 ^{A}	30.27 ± 1.47 ^{b,B}	33.07 ± 0.75 ^{A}	33.53 ± 0.78 ^{ab,A}	31.43 ± 0.45 ^{AB}	33.43 ± 1.15 ^{A}
2	32.97 ± 0.72 ^{AB}	33.60 ± 0.92 ^{a} .^{A}	34.03 ± 0.85 ^{A}	33.87 ± 0.76 ^{ab,A}	31.87 ± 0.71 ^{B}	33.20 ± 0.56 ^{AB}
3	31.70 ± 2.72 ^{AB}	30.20 ± 3.44 ^{b,B}	33.50 ± 0.50 ^{AB}	34.20 ± 0.98 ^{a,AB}	32.03 ± 0.90 ^{A}	34.63 ± 0.67 ^{A}
4	30.77 ± 3.20	32.63 ± 1.46 ^{ab}	33.93 ± 1.22	32.80 ± 0.75 ^{ab}	32.57 ± 1.40	33.53 ± 2.02
5	29.95 ± 0.07 ^{B}	34.90 ± 2.69 ^{a,A}	33.70 ± 0.28 ^{A}	33.15 ± 0.07 ^{ab,A}	32.35 ± 1.34 ^{AB}	34.15 ± 0.64 ^{A}
6	32.57 ± 0.21 ^{B}	32.83 ± 0.93 ^{ab,AB}	34.70 ± 0.20 ^{A}	34.47 ± 0.74 ^{a,AB}	32.57 ± 2.08 ^{B}	33.97 ± 1.08 ^{AB}
7	30.77 ± 2.35 ^{B}	32.57 ± 0.40 ^{ab,AB}	33.80 ± 1.40 ^{A}	33.87 ± 0.40 ^{ab,A}	33.80 ± 1.49 ^{A}	33.63 ± 1.56 ^{A}
8	31.80 ± 1.30	31.97 ± 1.10 ^{ab}	33.73 ± 2.46	32.07 ± 1.81 ^{b}	32.20 ± 1.64	34.03 ± 1.89

^{ab}Means with different superscript in each column differed significantly from each other (P<0.05)

^{AB} Means with different superscript in each low differed significantly from each other (P<0.05)

Table A. 8 Sperm pH of boar fed experiment diet (mean ± SD, n=3).

Diet	Time (Day)					
	-7	**1**	**7**	**14**	**21**	**28**
1	7.42 ± 0.24	7.38 ± 0.15	7.57 ± 0.09 ^{ab}	6.86 ± 0.04 ^{c}	7.05 ± 0.07 ^{b}	7.05 ± 0.18 ^{b}
2	7.41 ± 0.12 ^{A}	7.17 ± 0.07 ^{BC}	7.10 ± 0.14 ^{c,C}	7.19 ± 0.11 ^{b,BC}	7.29 ± 0.10 ^{ab,ABC}	7.37 ± 0.11 ^{ab,AB}
3	7.41 ± 0.07	7.33 ± 0.07	7.32 ± 0.04 ^{abc}	7.35 ± 0.08 ^{ab}	7.35 ± 0.08 ^{ab}	7.29 ± 0.12 ^{ab}
4	7.53 ± 0.22	7.34 ± 0.28	7.34 ± 0.34 ^{abc}	7.42 ± 0.29 ^{abc}	7.49 ± 0.21 ^{a}	7.36 ± 0.28 ^{a}
5	7.55 ± 0.06 ^{A}	7.37 ± 0.01 ^{C}	7.46 ± 0.02 ^{abc,B}	7.56 ± 0.00 ^{a,A}	7.50 ± 0.01 ^{a,AB}	7.51 ± 0.06 ^{a,AB}
6	7.37 ± 0.14	7.22 ± 0.11	7.21 ± 0.21 ^{bc}	7.22 ± 0.15 ^{b}	7.37 ± 0.22 ^{ab}	7.42 ± 0.23 ^{ab}
7	7.53 ± 0.14	7.30 ± 0.06	7.33 ± 0.17 ^{abc}	7.40 ± 0.18 ^{ab}	7.53 ± 0.26 ^{a}	7.51 ± 0.13 ^{a}
8	7.26 ± 0.24	7.23 ± 0.16	7.22 ± 0.14 ^{bc}	7.30 ± 0.20 ^{ab}	7.32 ± 0.18 ^{ab}	7.29 ± 0.25 ^{ab}

^{ab}Means with different superscript in each column differed significantly from each other (P<0.05)

^{AB} Means with different superscript in each low differed significantly from each other (P<0.05)

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High Selenium-Enriched Yeast Production

Prakaidoy Ditsayabut[1], Pakanit Kupittayanant[2], Chokchai Wanapu[1]
[1]*School of Biotechnology,* [2]*School of Animal Production Technology,*
Institute of Agricultural Technology,
Suranaree University of Technology, 111 University Avenue, Maung,
Nakhon Ratchasima 30000, Thailand
E-mail: prakaidoy_sut@hotmail.com, pakanit@sut.ac.th, wanapu@sut.ac.th

ABSTRACT

This paper describes a fermentation protocol for production of selenium-enriched yeast. *Saccharomyces bayanus* was selected because of its high Se accumulation. *S. bayanus* was tested and 1.075 mg/mL dry cell weight was obtained after 42 h. The organic Se was obtained 1 mg DWC by optimal Na_2SeO_3 addition for 20 mg/L. The organic Se was accumulated gradually with increasing DCW (6.43×10^{-3} mg/mg DCW) and the Se uptake level achieved 2.29×10^{-10} mg/cell yeast was achieved in a 5 L fermentor for 42 h cultivation.

Keywords: Fermentation, *Saccharomyces bayanus*, selenium-enriched yeast, sodium-selenite

INTRODUCTIONI

At high concentration, selenium (Se) is toxic and affects the central nervous system [1]. However, at low concentration, Se is an essential element for animal and human diets [2]. Se has structural and enzymatic roles as antioxidant and catalyst for the production of the active thyroid hormone; Se is needed for the proper functioning of the immune system and appears to be a key nutrient in inhibiting HIV progression to AIDS [3]. An elevated Se intake may be associated with reduced cancer risk. Se showed cancer protective effect and inhibition of the tumour cell invasion [4, 5]. It is generally believed that the ingestion of the organic Se compounds is better and safe than that of the inorganic Se. Some microorganisms produce biomass with high protein content and meanwhile transform inorganic Se (a low bio-availability, potentially toxic) into organic form (safe and highly bioactive). Organic Se complexes and Se containing amino acids are considered to be the most bio-available for human and animal [6]. Under appropriate conditions, yeasts are capable of accumulating large amounts of trace elements such as Se and incorporating them into organic compounds [7]. This mineral is absorbed during the yeast growth process. The ability of *Saccharomyces cerevisiae* to transform inorganic Se into organo-selenium compounds (especially selenomethionine) is known and it is dependent on growth conditions. Yeast is able to accumulate high concentrations of Se (3 mg/g) [8] and transform inorganic Se mainly into selenomethionine [9, 10]. Continuous fermentations in optimum medium of sodium selenite (Na_2SeO_3) demonstrated higher biomass and selenium content compared to the fermentation with sodium selenate (Na_2SeO_4) [11]. Yeast is known for its high protein content and therefore, more Se can be incorporated by replacement of sulphur in proteins compared with Se from plant sources. Also, the

BIOGRAPHY

Ms. Prakaidoy Sitsayabut was born on August 3, 1983 in Chiangrai, Thailand. She graduated with a bachelor degree of Animal Production of Technology, Suranaree University of Technology in Year 2006. After graduation, In 2007, she decided to study Master degree course in School of Biotechnology, Institute of Agricultural technology, Suranaree University of Technology with Asst. Prof. Dr. Chokchai Wanapu. Her master thesis topic was study of Organic selenium yeast production and its effects on boar sperm quality comparisons with inorganic selenium in diet. The results from some part of this study have been presented as poster presentation at the Burapha University International Conference, July 9-12, 2012 Pattaya, Thailand.