ผลของการเสริมซีลีเนี่ยมต่อคุณลักษณะการเจริญเติบโต ระดับธัยรอยด์ฮอร์โมน
เอนไซม์ต้านออกซิเดชัน และไดแซคคาริเดส ในลูกไก่กระทง

นางสาวฉัตรสุมาลย์ ศรีมงคล

EFFECTS OF SELENIUM SUPPLEMENTATION ON GROWTH
PERFORMANCE, THYROID HORMONE LEVELS, ANTIOXIDANT
ENZYME AND DISACCHARIDASE ACTIVITIES IN BROILER CHICK

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ฉัตรสุมาลย์ ศรีมงคล : ผลของการเสริมซีลีเนี่ยมต่อคุณลักษณะการเจริญเติบโต ระดับธัยรอยด์ฮอร์โมน เอนไซม์ต้านออกซิเดชัน และไดแซคคาริเดส ในลูกไก่กระทง (EFFECTS OF SELENIUM SUPPLEMENTATION ON GROWTH PERFORMANCE, THYROID HORMONE (T3) LEVELS, ANTIOXIDANT ENZYME AND DISACCHARIDASE ACTIVITIES IN BROILER CHICK) อ. ที่ปรึกษา ผศ.น.สพ.ดร. กฤษ อังคนาพร,อ.ที่ปรึกษาร่วม: รศ. สุวรรณา กิจภากรณ์; 74 หน้า. ISBN 974-17-4114-6

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมซีลีเนี่ยมและการอดอาหารต่อคุณลักษณะการเจริญเติบโต ระดับธัยรอยด์ฮอร์โมน เอนไซม์ต้านออกซิเดชัน และไดแซคคาริเดส ในลูกไก่กระทงโดยในการทดลองใช้ลูกไก่พันธุ์คือบ500 เพศผู้จากแม่ไก่ที่ไม่ได้เสริมซีลีเนี่ยม จำนวน 1,920 ตัว แบ่งออกเป็น 6 กลุ่ม กลุ่มที่ 12และ3ลูกไก่ได้รับอาหารทันทีที่มาถึงฟาร์มทดลอง โดยกลุ่มที่ 1 ได้รับอาหารพื้นฐานเป็นกลุ่มควบคุม กลุ่มที่ 2 และ3เสริมซีลีเนี่ยมอินทรีย์และอนินทรีย์ขนาด0.2 มิลลิกรัมต่อกิโลกรัมอาหารตามลำดับ กลุ่มที่ 4 5 และ6ลูกไก่ได้รับอาหารหลังจากที่มาถึงฟาร์มทดลอง 48 ชั่วโมง โดยได้รับอาหารเหมือนกับกลุ่มที่ 1 2และ3ตามลำดับ วันแรกของการทดลองทำการเก็บเลือดและตับจากลูกไก่10ตัวที่เหลือจากการจัดไก่เข้ากรง เพื่อใช้ในการหาระดับธัยรอยด์ฮอร์โมนและปริมาณเอนไซม์ต้านออกซิเดชันเป็นค่าพื้นฐาน และเมื่อลูกไก่อายุ 14-28 และ 42 วัน ทำการชั่งน้ำหนักไก่และบันทึกปริมาณอาหารที่กิน ซึ่งในวันที่ไก่อายุ 14 และ28 วัน ทำการสุ่มชั่งไก่รายตัวร้อย50 ในวันที่ไก่อายุ42วัน ชั่งไก่รายตัวทุกตัว และในวันที่ไก่อายุ 7 14 21 และ42 สุ่มไก่ทดลองกลุ่มละ8ตัวเก็บตัวอย่างเลือดและตับ เพื่อใช้ในการวิเคราะห์หาระดับธัยรอยด์ฮอร์โมนและปริมาณเอนไซม์ต้านออกซิเดชัน ในวันที่ไก่อายุ 21 วัน เก็บตัวอย่างเยื่อบุผนังลำไส้เล็กส่วนกลางมาตรวจวัดเอนไซม์ไดแซคคาริเดส

การเจริญเติบโตในภาพรวมตลอด 42 วันของการทดลอง พบว่าไก่ที่ได้รับอาหารทันทีมีน้ำหนักตัวที่เพิ่มขึ้น น้ำหนักตัวที่เพิ่มขึ้นเฉลี่ยต่อตัวต่อวัน ปริมาณการกินได้ต่อตัวและปริมาณการกินได้ต่อตัวต่อวันมากกว่าลูกไก่ที่อดอาหารอย่างมีนัยสำคัญ (p<0.05) แต่ไก่ที่อดอาหารมีประสิทธิภาพการเปลี่ยอาหารเป็นเนื้อที่ดีกว่าไก่ที่ได้รับอาหารทันทีอย่างมีนัยสำคัญ(p<0.05) และพบว่าไก่ที่ได้รับอาการที่เสริมเสริมซีลีเนี่ยมอินทรีย์มีน้ำหนักตัวสุดท้ายและความสม่ำเสมอของฝูงสูงกว่ากลุ่มที่ได้รับซีลีเนี่ยมอนินทรีย์ และพบว่าการเสริมซีลีเนียมช่วยเพิ่มระดับเอนไซม์กลูตาไธโอนในวันที่ 7 และ 42 และยังเพิ่มระดับเอนไซม์ซุปเปอร์ออกไซด์ ดิสมิวเตสได้ในวันที่ 14 และ42และนอกจากนี้ยังพบว่า ไก่ที่อดอาหารที่อายุ7วัน มีระดับธัยรอยด์ฮอร์โมนสูงกว่ากลุ่มที่ได้รับอาหารทันทีและกลุ่มที่ได้รับการเสริมซีลีเนี่ยมมีระดับธัยรอยด์ฮอร์โมนสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ(p<0.05) และมีแนวโน้มเพิ่มปริมาณเอนไซม์ไดแซคคาริเดส (อายุ 21 วัน) และพบว่าการเสริมซีลีเนี่ยมและการอดอาหารไม่มีผลต่อระดับเอนไซม์คะตาเลส

จากการศึกษาครั้งนี้สรุปได้ว่า การอดอาหารลูกไก่ 48 ชั่วโมงหลังฟัก ทำให้น้ำหนักตัว การกินได้ และความสม่ำเสมอของฝูงลดลง แต่ช่วยให้ประสิทธิภาพการเปลี่ยนอาหารเป็นเนื้อได้ดีขึ้นและช่วยลดอัตราการตาย นอกจากนี้การเสริมซีลีเนี่ยมในลูกไก่ทั้งที่ได้รับอาหารทันทีและลูกไก่ที่ถูกอดอาหาร ช่วยเพิ่มปริมาณเอนไซม์กลูตาไธโอนเปอร์ออกซิเดส (ที่อายุ 7และ14 วัน) ซุปเปอร์ออกไซด์ดิสมิวเตส (ที่อายุ 14 และ 42 วัน) ระดับไตรไอโอโดธัยโรนีนที่อายุสองสัปดาห์ และมีแนวโน้มเพิ่มระดับไดแซคคาริเดสได้ การเสริมซีลีเนี่ยมอินทรีย์ช่วยให้คุณลักษณะการเจริญเติบโตดีขึ้น (น้ำหนักตัวที่เพิ่มขึ้นเฉลี่ยต่อตัวต่อวัน และประสิทธิภาพการเปลี่ยนอาหารเป็นเนื้อ ที่อายุ 15-28 วัน) และความสม่ำเสมอของฝูง (ที่อายุ 1-42 วัน)ได้ดีกว่าการเสริมซีลีเนี่ยมอนินทรีย์

ภาควิชา สรีรวิทยา	ลายมือชื่อนิสิต...
สาขาวิชา สรีรวิทยาการสัตว์	ลายมือชื่ออาจารย์ที่ปรึกษา......................................
ปีการศึกษา 2546	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม................................

The objectives of this investigation were to study the effect of selenium supplementation and fasting on growth performance and flock uniformity, thyroid hormone (T3) levels, antioxidant enzyme and disaccharidase activities of broiler chicks. One thousand nine hundred and twenty, day old, male chicks (Cobb 500) from parent stock without selenium supplementation in diet were allocated into 6 treatments. The treatment were T1: chicks were fed immediately after arrival and received basal diet, T2 and T3 chicks were fed immediately after arrival and received basal diet plus 0.2 ppm as organic selenium and 0.2 ppm as inorganic selenium, respectively. T4, T5 and T6 chicks were fasted for 48 h after arrival and received diets as same as T1, T2 and T3, respectively. At days 14, 28 and 42 of age, body weight and feed intake were recorded. Individual body weight was measured from 50 % of chicks in each treatment at days 14 and 28 of age and 100 % of chicks in each treatment at days 42 for uniformity calculation. On the first day, ten extra chicks were collected for blood samples from heart for background thyroid hormone (T3) levels. Chicks were killed using carbon dioxide inhalation chamber, liver samples were collected for background antioxidant enzyme activities. On days 7, 14, 21 and 42, one chicks from each replicate pen was selected and blood and liver sample were collected for determine thyroid hormone levels and antioxidant enzyme activities. At days 21, jejunum from each chick was excised and mucosa was scraped for determination of disaccharidase activities.

At the overall period (days 1-42 of age), fed chicks had significantly (p<0.05) higher body weight gain, ADG, feed intake and DFI than fasted chicks. On the contrary, fasted chicks had significantly (p<0.05) better FCR than fed chicks. Chicks that received diet supplemented with OS had the highest final body weight and body weight uniformity. In addition, selenium supplemented chicks had significantly (p<0.05) higher GSH-Px (days 7 and 42 of age) and SOD (days 14 and 42 of age) activity than control. It was found that fed chicks and selenium supplemented chicks had significantly (p<0.05) higher T3 levels than fasted chicks and control. There were no significant difference on CAT (days 1-42) and disaccharidase activity (days 21) from treatment diet.

In conclusion, fasting chicks for 48 h reduced body weight, feed intake and flock uniformity, but these chicks had better FCR and mortality rate compared to fed chicks. Selenium supplementation in both fed and fasted chicks helped to increase GSH-Px (days 7 and 42 of age) and SOD 14 and 42 of age, enhance T3 levels in the second week, trended to increase disaccharidased at days 21 of age and improve growth performance. Organic selenium supplemented chicks had better growth performance (ADG, FCR at days 15-28 of age and flock uniformity at days 1-42 of age) and uniformity than inorganic selenium supplemented chicks.

Department Physiology	Student's signature………………………….
Field of study Animal Physiology	Advisor's signature………………………….
Academic year 2003	Co-advisor's signature………………………

ACKNOWLEDGEMENT

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ABBREVIATION

ADG	=	average daily gain
ANOVA	=	Analysis of Variance
BSA	=	bovine serum albumin
CAT	=	catalase
cm	=	centimeter
df	=	degree of freedom
DFI	=	daily feed intake
FCR	=	feed conversion ratio
gm	=	gram
GSH-Px	=	glutathione peroxidase
h	=	hour
IOS	=	inorganic selenium
kg	=	kilogram
wt	=	weight
min	=	minute
mg	=	milligram
mg %	=	milligram percent
mM	=	millimolar
mol	=	mole
no	=	number
OS	=	organic selenium
ppm	=	part per million
ROS	=	Reactive Oxygen Species
Sec	=	second
Se	=	selenium
U	=	unit
UV	=	Ultra Violet
U/ml	=	unit per milliliter
U/mg protein	=	unit per milligram protein
μmol	=	micromole

µM	=	micromolar
µg	=	microgram
µl	=	microliter

CHAPTER I

INTRODUCTION AND AIM

Broiler production in Thailand has expanded markedly during this decade. Management of early broiler nutrition is very important when chicks are transported from hatchery to farm. Since chicken embryos have wide and variable time of hatching, commercial hatcheries do not remove chicks until the maximum number of eggs hatched. Thus, at exit from hatchery, the chicks had average more than 1 day of age (Moran and Reinhart, 1980). Hatchery treatment such as debeaking, vaccination, sexing and transport to farms results in an additional time lag before birds are given first access to feed and water. Thus, most chicks were fasted for 48 h or more before the first access to feed. Previous studies have indicated that this process results in a decrease in growth in the post-hatch day and lower body weights and proportion of breast muscle at marketing (Noy et al, 1996). In addition, delay feeding in the first few days of life reduces final body weight (Noy and Sklan, 1999) and insufficient intake of feed will considerably affect feed conversion ratio, average daily gain, feed intake, mortality rate and uniformity of chicks in each flock (Geyra et al, 2001).

Following hatching in chicks, an abrupt change occurs in the source of nutrients as yolk is replaced by exogenous diet rich in carbohydrates and protein. Intake of exogenous feed is accompanied by rapid development of the gastrointestinal tract and associated organs to assimilate the ingested nutrients (Uni et al, 1998). The small intestines of hatching chicks underwent rapid development changes in the immediate 48 h post-hatch when the birds are transition from commercial hatcheries to farms (Geyra et al., 2001). All intestinal epithelial cells were proliferating at hatch, which changed rapidly within 48 hr posthatch (Noy et al, 2001). Lack of access to feed and water immediate transport depressed the rate of growth of villi and enterocyte length in all intestinal segments until 6 days posthatch. Tri-iodothyronine (T3) concentration may mediate some of the intestinal effects of feed deprivation. Plasma concentrations of T3 were decreased in unfed chicks (Noy and Sklan, 2001).

In addition, T3 influences growth, differentiation and maturation of birds. A requirement for thyroid hormones in the growth of birds had been demonstrated by the reduced growth that results from thyroidectomy or goitrogen administration. Thyroid

hormones were important in triggering tissue specific differentiation and maturation processes in many tissues (Mcnabb, 2000). Moreover, thyroid hormone especially T3 which affects early broiler growth, were linked with body selenium. Transformation of T4 to T3 required deiodination in the liver which is operated by a selenium enzyme, deiodinase (Jensen *et al*, 1986; Beckett *et al*, 1987; Underwood and Suttle, 1999).

Moreover, thyroid hormones influence metabolic rate, as the development of the avian embryo is dependent on aerobic metabolism. The rate of oxygen consumption increases dramatically from about the mid-period of the 21-days development. Such increase in mitochondrial respiration and oxygen uptake are obligatory aspects of embryonic development, providing the energy for tissue growth and transportation of nutrients. However, it is likely that beneficial aspects may be accompanied by potentially harmful effects because high rate of energy metabolism can lead to production of reactive oxygen species and other free radicals which can cause damage to cellular macromolecules (Noble *et al*, 1993). Tissues of newly hatched chicks express a range of antioxidant defence including natural antioxidant and antioxidant enzymes (Surai *et al*, 1999a; Surai, 2000). Antioxidant enzyme activities such as, glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD), were found to decrease during hatch (Surai, 1999).

Maternal diet composition is a major determinant of antioxidant system development in the chicks during embryogenesis and in early postnatal development. It was found that nutritional status of the laying hen determined the efficiency of the antioxidant system throughout early postnatal development of the offspring. An optimal antioxidant status of the newly hatched chick was effective for protection against damaging effects of free radicals and products of their metabolism. Chicks from hen that did not receive diet supplemented with organic selenium, had decreased antioxidant enzyme activity and increased liver lipid peroxidation (Surai, 2000).

The hypothesis of this study was that supplementing selenium in fasted chicks, from parent stock which were not supplemented with organic selenium, at early age can ameliorate the adverse effect of low antioxidant activity, thyroid hormone levels and small intestinal mucosal growth. This effect will subsequently lead to better growth performance and body weight uniformity of broilers.

The objectives of this experiment were to examine:

1. Effects of posthatch feeding and selenium supplementation on growth performance, thyroid hormone (T3) levels, antioxidant enzyme and disaccharidase activities.

2. Effect of organic selenium supplementation in comparison to inorganic selenium supplementation on growth performance, thyroid hormone (T3) levels, antioxidant enzyme and disaccharidase activities.

CHAPTER II

BACKGROUND INFORMATION

1 Selenium

For many years, biological interest in selenium was confined to its toxic effects on animals. Until 1957 selenium was discovered that it played an essential physiological role in the animals, despite being present at lower concentrations in the tissue than other essential elements (Scott *et al*, 1982).

1.1 Properties of selenium compounds

The properties of selenium are very similar to those of sulfur and tellurium. Selenium was found in association with sulfur in both inorganic and organic compounds.Selenium can replace sulfur in some compound and selenium was also found as a complex to sulfur apparently by coordinate covalent bonding. The most common inorganic forms of selenium are selenic acid, selenous acid, sulfurous acid, selenate and selenite (Scott *et al*, 1982).In plant and microorganisms, selenium had been shown to be able to replace sulfur in cysteine and methionine, thereby producing organic selenoprotein (selenocystine and selenomethionine) (Rayman, 2002).

1.2 Metabolism

Absorption and retention of selenium depend on its dietary form. Inorganic selenium (IOS), selenite and selenate ion are absorbed easily using the facilitated transport system from small intestine, but most of them rapidly excreted via the urine. IOS can not be converted to selenomethionine and only small amount of IOS found in body selenoprotein. While organic selenium (OS), selenomethione and selenocysteine are absorbed from the small intestine by the same natural amino acid transport and can be retained in the animal body longer than the inorganic form and it is storage form in liver and muscle (Scott *et al*, 1982).

1.2.1 Incorporation into selenoprotein

The specialized selenoproteins are formed in the liver and other tissues when specific selenocysteine and selenomethionine residues were incorporated into peptide chains. While selenoproteins contain a selenocysteine residue at the active site, this selenocysteine was biosynthezised in the body rather than absorbed directly from the diet because it was not a stable amino acid, that is why it is storage as selenomethione . All dietary selenomethionine mobilized from tissue storage was first converted to hydrogen selenide (H_2Se), then to selenophosphate followed by selenocysteine and selenoprotein (Underwood and Suttle, 1999).

1.2.2 Cellular uptake

Occurrence of selenium in tissues were varied with species and organ. The richest tissue is kidney, it contains 15-20 fold higher concentrations than the poorest tissue, muscle. There are corresponding differences in enzyme activities of the principal selenoprotein present. For example, in the chick and rat, the liver and erythrocyte have the highest GSH-Px1 activity. Selenium intake dramatically affects the GSH-Px1 activity of body components and also the relative body distribution of the enzyme. Other GSH-Px also show contrasting tissue responses to selenium supplementation (Lei *et al*, 1998).

1.2.3 Excretion

Selenium can be lost from the body by exhalation, urinary excretion or fecal endogenous excretion. Biliary secretion of selenium can amount to 28 % of intake, although most was reabsorbed (Underwood and Suttle, 1999).

1.3 Functions of selenium

Selenium is necessary for growth (Surai, 1999; Jianhua *et al*, 2000), prevention of a variety of disease condition (Noguchi *et al*, 1973; Smart *et al*, 1985) and reduction

of mortality rate in chicks (Colnago *et al*, 1982). In addition, the organic selenium as selenoprotein acts as antioxidant. The four known glutathione peroxidases (GSH-Px1, GSH-Px2, GSH-Px3 and GSH-Px4) utilize as reducing substrate and they are important in controlling peroxidation, which can lead to chain reactions of free radicals generation and tissue damage. The task of terminating such reaction and protecting against peroxidation is shared by other tissue enzymes (e.g. the superoxide dismutase, copper-zinc (Cu Zn) and manganese (Mn) SOD; catalase; glutathione-sulphur(S)-transferase) and by non-enzyme scavengers, such as vitamin E (Surai *et al*, 1999a). Selenium deficiency caused peroxidative damage, that was depended on the degree of free radical generation (Surai *et al*, 1999b).

1.4 Selenium Toxicity

Selenium is the most toxic of the essential trace element when used in high dose (at least 10-fold of physiological requirements). One of the mechanism by which selenium exerts its toxic effects in animals appears to be through its competition with sulfur compound or because of its strong affinity for the sulfur-selenium complexes. In chickens, excessive levels of selenium reduced growth rate, egg production, hatchability and caused embryonic abnormalities (Scott *et al*, 1982). In previous study, chicken were fed a basal diet containing 0.3 mg Se/kg diet and supplemented with 0, 0.1, 0.5, 1.0, 3.0 and 6.0 mg/kg in organic form (Selenomethionine) for 18 weeks, it was found that no toxic effect. On the contrary, selenium supplementation with 0.1 up to 9 mg/kg diet as sodium selenite in Leghorn, hatchability of fertile egg, egg weight and egg production were significantly decreased by 4-9 mg/kg diet (Surai, 2002).

2 Selenoprotein function

2.1 Cytosolic peroxidase

The first peroxidase to be identified and studied in detail is known as cytosolic peroxidase or GSH-Px1. It is the predominant GSH-Px and source of selenium in erythrocyte and liver, and all selenium responsive diseases are accompanied by decrease

in blood and tissue GSH-Px1 activities. Selenium is present in GSH-Px1 in stoichometric amounts, with 4g atoms Se per mol and the tetrameric enzyme catalyzes the reduction of hydrogen peroxide (H_2O_2) and prevent hydrogen peroxide formed from fatty acids and other substances.
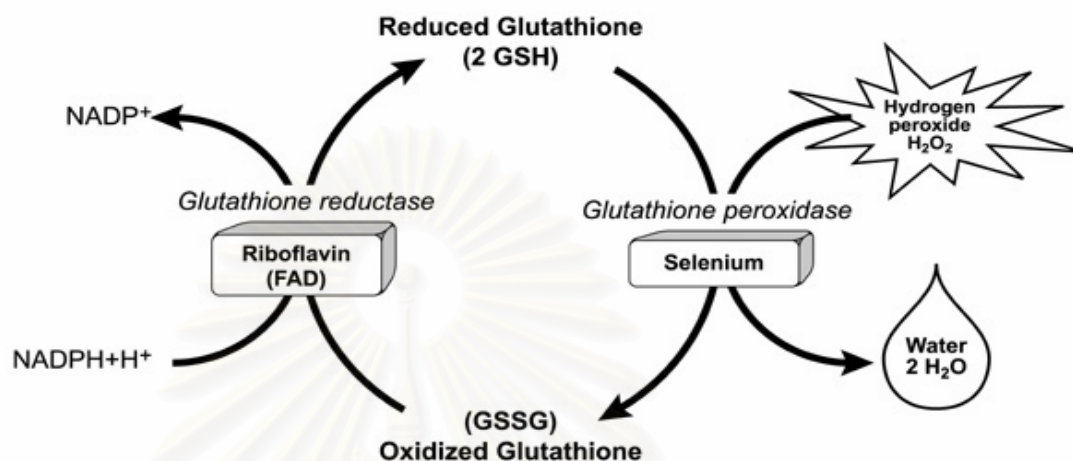
Figure 1 Important reactions showing mechanism whereby glutathione peroxidase destroys peroxides.

Figure 1 depicts the glutathione oxidation-reduction cycle. One molecule of hydrogen peroxide is reduced to two molecules of water, while two molecules of reduced glutathione (GSH) are oxidized in a reaction catalyzed by the selenoprotein enzyme, glutathione peroxidase. Oxidized glutathione may be reduced by the flavin adenine dinucleotide (FAD) dependent enzyme, glutathione reductase.

2.2 Gastrointestinal peroxidase

The gastrointestinal peroxidase (GSH-Px4) may act locally to protect the intestinal mucosa from dietary hydroperoxide (Chu *et al*, 1993).

2.3 Other peroxidases

The plasma or extracellular peroxidase, GSH-Px2 and phospholipid peroxidase or intracellular peroxidase, GSH-Px3 were tetrameric and synthesized principally in the

lung and kidney, where their main functions may reside in protecting the renal proximal tubule from peroxidative damage (Underwood and Suttle, 1999).

2.4 Deiodinase

The first indication of selenium deficiency in iodine metabolism came from an increase in the tetra to tri-iodothyronine (T4:T3) ratio in selenium depleted rats (Arthur *et al*, 1990; Beckett *et al*, 1992). Eventually, a membrane bound selenoprotein was defined as type I iodothyronine deiodinase (ID1) which was capable of transforming T4 to the physiologically active form, T3 (Figure 2) (Beckett *et al*, 1987; Jensen *et al*, 1986; Underwood and Suttle, 1999). This deiodinase is located primarily in the liver and kidney and none is present in the thyroid gland of farm livestock. A second deiodinase (ID2) can also form T3 from T4, but under feedback control from T4 and therefore liable to be doubly inhibited in selenium deficiency. ID2 is most abundant in brain and brown adipose tissue (BAT) and thermogenesis in the newborn is dependent on BAT and T3 generation. The thyroid hormones play important roles in growth and protein turnover (Hayashi *et al*, 1985) therefore, selenium deficiency might affect protein turnover followed by growth retardation, as T3 production was impaired (Jianhua *et al*, 2000).
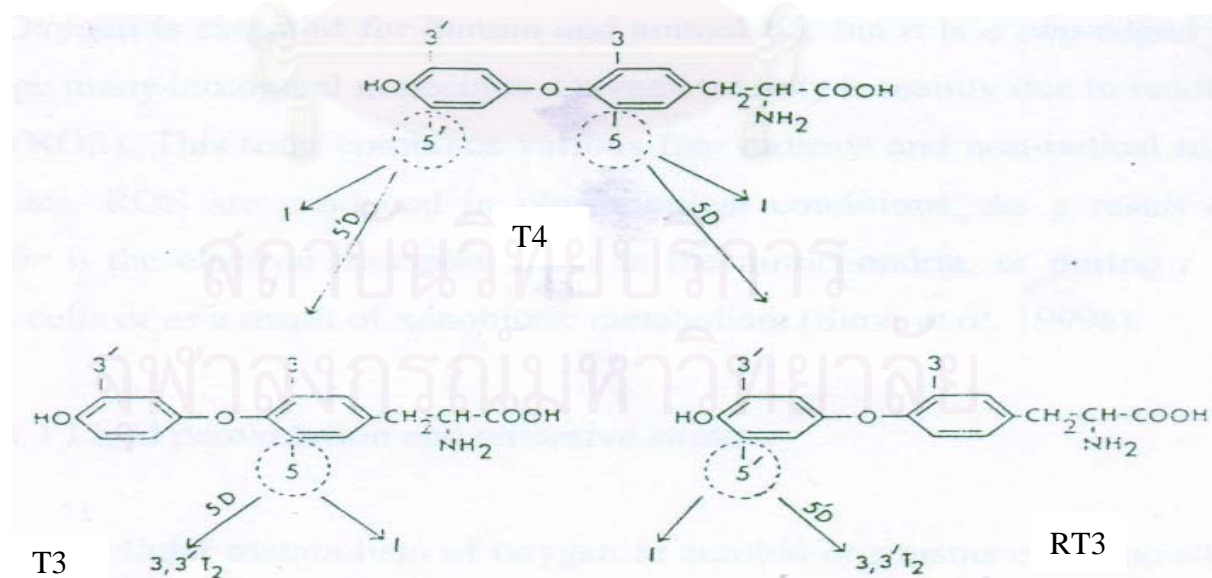
Figure 2 Thyroid hormone conversions by deiodinations. Numbers on the ring indicate the positions of iodine atom. T4, thyroxine; T3, tri-iodothyronine; rT3, reverse-tri-iodothyronine.

3 Effects of thyroid hormone on growth and development.

The thyroid hormones are necessary for growth, development and they play a major role in regulating oxidative metabolism of birds. In addition, thyroid hormones are important in hatching. They stimulated a variety of development and metabolic processes necessary for successful hatching. Thyroid hormone action is mediated through nuclear receptor. These receptors are referred to as tri-iodothyronine (T3) receptors because they bind T3 with higher affinity than they bind thyroxine (T4) or any other functional thyroid hormone analog. The primary direct hormonal stimulation of body growth results from circulating growth factors. Likewise, altered thyroid hormone concentration influence the metabolic energy supply, liver glycogen storage is facilitated by increase thyroid hormone (Mcnabb, 2000). Moreover, T3 concentrations may mediate some of the intestinal effects of delayed fed chicks that caused decrease plasma T3 concentrations (Noy *et al*, 2001).

4 Effects of reactive oxygen species and antioxidant defense in animal health.

Oxygen is essential for human and animal life but it is a two-edged sword able to damage many biological molecules. Oxygen toxicity is mainly due to reactive oxygen species (ROS). This term combines various free radicals and non-radical toxic oxygen metabolites. ROS are produced in physiological conditions. As a result of electron escape from the electron transport chain in the mitochondria, or during activation of immune cells.(Surai *et al*, 1999a).

4.1 Lipid peroxidation and oxidative stress

The cellular metabolism of oxygen in aerobic organisms continuously produces small amounts of ROS and large increase amount of three reactive species lead to oxidative stress, which was defined as a disturbance in favor of the pro-oxidants-antioxidant balance in favor of pro-oxidants leading to potential damage. Oxidative stress may be influenced by a number of different factors such as genetic, age,

nutritional status, environment, drug composition and medication. Under normal circumstance, the major source of ROS produced in the body is electron leakage from mitochondrial and microsomal electron transport chain. However, phagocytic cells may be an important source of ROS. Lipid peroxidation, as a mechanism of oxidative stress can cause damage to cell membranes directly by altering membrane fluidity, permeability or integrity. Lipid peroxide can form cyclic peroxide, which can decomposes to highly cytotoxic products. These compounds react aggressively with living tissue and can disrupt cell membrane structure and diffuse from the lipid membrane and damage other cells (Surai *et al*, 1999a).

4.2 Antioxidant defenses against oxidative stress.

ROS induced oxidative stress was rigorously controlled by multiple defensive lines that include enzymatic and non-enzymatic scavengers (vitamin A, E, C and glutathione) and quenchers. These defense either stop the free radical chain reaction or divert the free radical to a less deleterious target. The first line of defense consists of mineral-dependent enzyme groups responsible for controlling ROS such as superoxide dismutase (SOD), which scavenges superoxide anion in the mitochondria, nucleus and cytoplasm. The catalase (CAT), a Fe containing enzyme, can remove . The glutathione peroxidase (GSH-Px), a Se containing enzyme, can convert H_2O_2 to water and oxygen. The relationships among antioxidant enzymes are shown in Figure 3. The SOD is required for H_2O_2 production and GSH-Px or CAT are needed for converting reactive oxygen species to water.
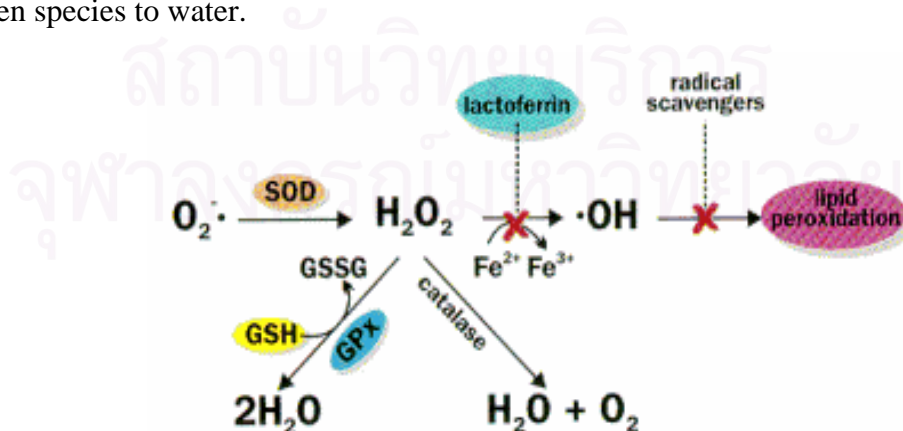
Figure 3. Relationships among antioxidant enzymes

The first antioxidant defense was not sufficient to completely prevent free radical formation and lipid peroxidation, resulting in some ROS escape. Therefore a second level of defense, comprises chain breaking antioxidant, can be stimulated to action. Chain breaking antioxidant inhibit peroxidation by keeping the chain length of the propagation reaction as small as possible. Though, a second level of antioxidant defense in the cell was not sufficient to prevent lipid peroxidation and destruction of some biological molecules. The third level of defense, consist of system that eliminate or repair damaged molecules will further take action. The mechanism by which these antioxidants act at the molecular and cellular level include roles in gene expression and regulation, apoptosis and signal transduction. Thus, these antioxidant molecules are involved in fundamental metabolic and homeostatic processes (Surai *et al*, 1999a). The intracellular antioxidant defense system is shown in Figure 4.
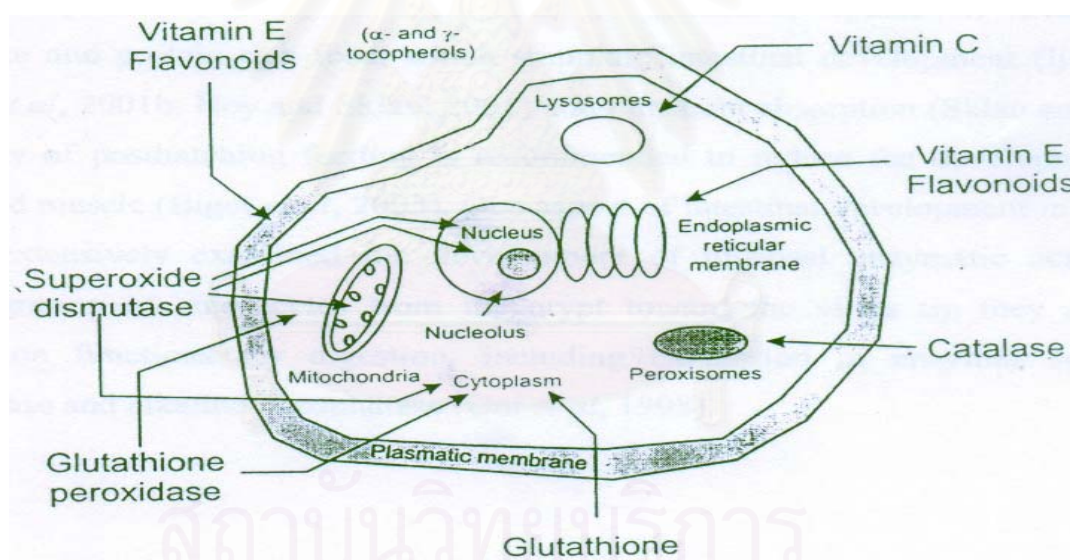
Figure 4 Intracellular organization of the antioxidant defense system.

5 Effects of delayed feed intake on growth performance.

At present broilers reach slaughter weight physiologically younger and first week after hatching represents a larger proportion of whole life span (Bigot *et al*, 2003). Following hatching in birds, an abrupt change occurs in the source of nutrients as yolk was replaced by exogenous diet. Nutrients in the residual yolk not being used during fetal life are supposed to supply the lack of food during the fasting period. However, an insufficient contribution to the nutritional requirement for both maintenance and growth in today' s broiler chicks occurred (Noy and Sklan, 2001). In practice, hatching and transportation procedures delay the feeding of chicks by 10 to 60 h. Delay feeding in the first few days of life reduces final body weight (Noy *et al*, 2001). Intake of exogenous feed was accompanied by rapid development of the gastrointestinal tract and associated organs to assimilate to ingested nutrients (Uni *et al*, 1998; Geyra and Uni, 2001). In addition, once exogenous feed intake commences, chicks must undergo metabolic adaptation from yolk dependent to nutritional uptake of exogenous carbohydrate and protein rich feed, which stimulates intestinal development (Iji *et al*, 2001a; Iji *et al*, 2001b; Noy and Sklan, 2001) and nutrient absorption (Sklan and Noy, 2000). Delayed of posthatching feeding was found to reduce the development of intestine and muscle (Bigot *et al*, 2003). One aspect of intestinal development in chicks that has been extensively examined is the development of mucosal enzymatic activities. During migration of enterocytes from the crypt toward the villus tip, they acquire differentiation functions for digestion, including expression of enzymes such as disaccharidase and alkaline phosphatase (Uni *et al*, 1998).

CHAPTER III

MATERIALS AND METHODS

1 Animals and Diets

One thousand nine hundred and twenty (1,920) male, day-old broiler chicks (Cobb 500), were used in a 2 x 3 factorial arrangement in completely randomized design. The variables included feeding regimen (fed immediately and fed at 48 hr after chicks arriving at the farm), and selenium supplementation (no Se supplementation, supplementation with 0.2 ppm Se as organic selenium (OS), and supplementation with 0.2 ppm Se as inorganic selenium (IOS). These chicks were obtained from the parent stock that had never been supplemented with organic source of selenium. Each of the six treatment combinations were replicated eight times using 40 chicks per pen.

The composition and nutrient contents of the basal diets are shown in Table 1. The starter, grower and finisher diets were used when chicks are 0-14, 15-28 and 29-42 days old, respectively. The difference of each treatment was based on the absence (treatments 1 and 4) or presence of selenium as OS (treatments 2 and 5) or IOS (treatments 3 and 6) in the vitamin mineral premix. The diet composition from proximate analysis and selenium concentration are shown in Tables 2 and 3, respectively. The experiment was conducted in a closed concrete-floor-pen house with evaporative cooling facility. Each pen measured at 2 m x 2 m and was equipped with a self feeder and four nipple water drinkers. Chicks arrived at the experimental farm within 5 hr of transportation from hatchery farm in Nakorn Ratchasima province. Feed and water were provided *ad libitum*. All experimental diets were pelleted. Lighting was provided continuously with light intensity of 20 lux in the first week, 15 lux in second week, 10 lux in third week and 5 lux fourth week until in the last week. All birds were vaccinated with Newcastle and Infectious Bronchitis diseases at 10 days of age and Gumboro disease at 14 days old. The average max/min temperature and relative humidity in broilers house were 32.5/30.4 ^{o}C / 72.6%, 30.3/28.7 ^{o}C / 87.4% and 30.3/28.1 ^{o}C / 89.6% during starter, grower and finisher periods, respectively.

Table 1 Composition and nutrient content of basal diets

Ingredients		Starter	Grower	Finisher
Corn 7.5%	%	52.133	51.799	56.681
Soybean meal, 48.7%	%	25.397	24.060	17.876
Full fat soybean, 36.6%	%	15.000	15.000	15.000
Fatpak 100 (Dry fat)	%	1.500	1.500	1.500
Palm oil	%	1.212	2.938	4.133
L-Lysine HCl	%	0.224	0.225	0.255
DL-Methionine	%	0.384	0.403	0.422
L-Threonine	%	0.094	0.090	0.126
Monodicalcium phosphate	%	1.875	1.890	1.945
Limestone 38.18%	%	1.318	1.324	1.351
Sodiumbicarbonate, 27% Na	%	0.150	0.100	0.050
Salt	%	0.343	0.302	0.289
Choline chloride 60%	%	0.100	0.100	0.100
Vitamin premix[1]/mineral premix [2]	%	0.150	0.150	0.150
Filler (corn starch)	%	0.020	0.020	0.020
MTB 100 (mycotoxin binder)	%	0.050	0.050	0.050
Sacox120 (12% salinomycin sodium)	%	0.050	0.050	0.050
Total batch		100.00	100.00	100.00
Nutrients				
Weight	Kg	1	1	1
Dry matter	%	88.70	89.06	89.13
ME for poultry	Kcal/kg	3,070	3,166	3,286
Crude protein	%	22.26	21.60	19.02
Crude fat	%	7.74	9.42	10.70
Linoleic acid	%	2.21	2.38	2.56
Crude fiber	%	3.79	3.69	3.36
Lysine	%	1.40	1.36	1.21
Arginine	%	1.49	1.44	1.24
Methionine	%	0.70	0.71	0.70
Met + Cys	%	1.04	1.04	0.99
Threonine	%	0.94	0.91	0.84
Tryptophan	%	0.27	0.26	0.23
Isoleucine	%	0.94	0.91	0.79
Leucine	%	1.84	1.78	1.60
Valine	%	1.07	1.04	0.94
Calcium	%	0.90	0.90	0.90
Phosphorus-total	%	0.79	0.79	0.77
Phosphorus-avail.	%	0.45	0.45	0.45
Potassium	%	0.91	0.88	0.78
Sodium	%	0.20	0.17	0.15
Chloride	%	0.24	0.22	0.21

[1] Content per 1kg vitamin premix: vitamin A, 80 MIU; vitamin D3, 6.7 MIU; vitamin E, 100 gm; vitamin K3, 7 gm; vitamin B1, 10 gm; vitamin B2, 20 gm; vitamin B6, 13 gm; vitamin B12, 0.12 gm; pantothenic acid, 50 gm; niacin, 100 gm; folic acid, 3 gm; biotin, 0.3 gm; preservative, 2.5 gm; anti-caking, 10 gm; wheat middling, 684.08 gm.

[2] Content per 1 kg mineral premix: Fe, 40 gm; Mn, 90 gm; Zn, 80 gm; Cu, 85 gm; I, 1 gm; calcium carbonate, 704 gm.

Table 2 Composition of diet from proximate analysis

Item	Starter diet	Grower diet	Finisher diet
		(%)	
Moisture	10.32	8.89	8.99
Protein	21.39	20.27	18.31
Fat	7.60	10.59	10.61
Fiber	2.77	2.48	2.56

Table 3 Selenium concentration in experimental diet from fluorometric method.

Diet	Starter diet	Grower diet	Finisher diet
		Se concentration (ppm)	
Basal diet	0.148	0.129	0.161
Basal diet plus OS	0.323	0.337	0.247
Basal diet plus IOS	0.323	0.326	0.273

Experimental procedure

This experiment was designed for chicks in the same condition, 1,920 chicks were allocated into 6 groups of 320 chicks (8 replicates of 40 chicks each).

Group 1.Chicks were fed immediately after arrival and received basal diet .

Group 2.Chicks were fed immediately after arrival and received basal diet and plus 0.2 ppm as OS.

Group 3. Chicks were fed immediately after arrival and received basal diet and plus 0.2 ppm as IOS.

Group 4.Chicks were fasted 48 hr after arrival and received basal diet.

Group 5.Chicks were fasted for 48 hr after arrival and received basal diet and plus 0.2 ppm as OS.

Group 6. Chicks were fasted for 48 hr after arrival and received basal diet and plus 0.2 ppm as IOS.

2 Protocol of experiment

\...........................treatment period...............................\

1	7	14	21	28	42 days of age
ls	ls	ls	ls	-	ls
bs	bs	bs	bs	-	-
-	-	-	ms	-	-
w	-	w	-	w	w
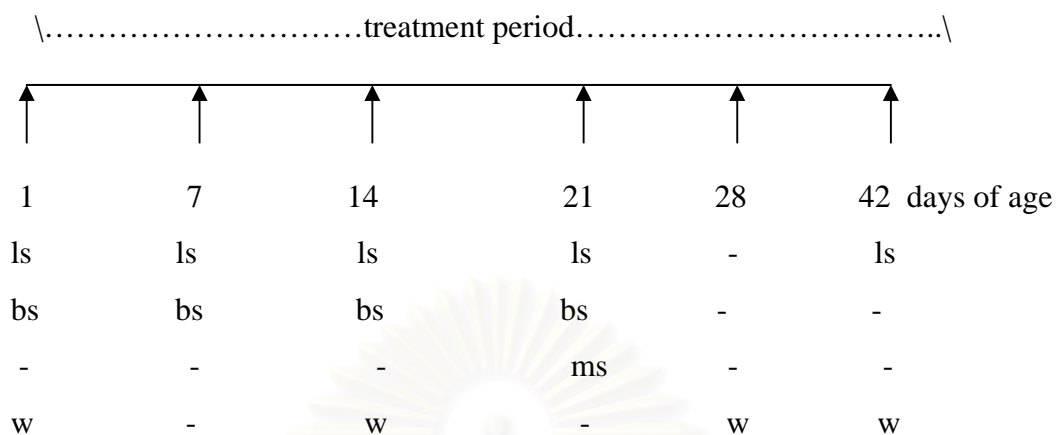
ls = liver sampling

bs = blood sampling

ms = mucosal cell sampling

w = weighing

Figure 5 Diagram showing the whole period of the experiment.

3 Sample collection and tissue preparation

Total pen feed consumption was recorded weekly. Sample collection days are shown in Figure 5. Body weight as pen basis was measured for growth calculation on days 1, 14, 28 and 42 of age. Individual body weight was measured from 50% of chicks in each pen for chick uniformity calculation on days 14 and 28, and 100% of chicks on day 42. Mortality and culling were recorded when occur, culled chicks were base on lower standard body weight or weakness. On the first day, blood samples from ten chicks were collected from heart into eppendorf tubes for background thyroid hormone (T3) levels. All chicks were euthanased using carbon dioxide inhalation chamber, liver samples were collected into plastic pouch and stored at –20 °C until analyzed for background antioxidant enzyme activities. On days 7, 14, 21 and 42, one chick from each replicate pen was randomly selected and blood samples were collected from wing vein into an eppendorf tube and euthanased for liver sample collection and stored at –20 °C until analyzed. Blood samples were centrifuged at 3,500 rpm (Centrifuge, GLC-2B, SoRVALL), for 15 min and serum samples were collected and stored at –20°C until analyzed. Serum thyroid hormone (T3) levels were analyzed by chemiluminescence immunoassay. Liver samples were washed in potassium phosphate buffer and homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) in nine volume (to determine GSH-Px) or four volume (to determine SOD) of same buffer. The homogenate was centrifuged at 3,500 rpm, for 30 minutes and the supernatant was used to determine GSH-Px activity (Tappel, 1978; Armstrong, 1998) and SOD activity (Mccord and Fridovich, 1969). Liver samples were homogenized with four part of detergent (1% Triton X-100) for determination of CAT activity (Beers and Sizer, 1951; Aebi, 1984). At day 21, jejunum from each chick was excised and mucosa was scraped for determination of disaccharidase activities (Dalhquist, 1968). Each intestinal section was opened longitudinally, rinsed with ice cold saline and placed on foam pad. Mucosal samples were scraped using a glass slide, wrapped with aluminium foil and stored at –70° C until analyzed.

4 Determination of mucosal and liver protein concentrations

4.1 Tissue preparation

Total protein concentrations in jejunal mucosa and liver were determined using Lowry method (1951). Jejunal mucosal scrapings were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four parts of distilled water, weight by volume (w/v). Then the homogenates were diluted 40 times with distilled water, volume by volume (v/v). Liver samples were homogenized with ten parts of distilled water, w/v. Then the homogenates were diluted 20 times with distilled water, v/v.

4.2 Assay procedure

The test tube containing 100 μl of the homogenate samples were added with 3.0 ml of fresh reagent (consist of 50 ml of 2% Na_2CO_3 in 0.1 M NaOH, 0.5 ml of 1% $CuSO_4 \bullet 5H_2O$ and 0.5 ml of 2% Na-tartrate). Then the test tubes were allowed to settle at room temperature for 10 min. Folin reagent (300 μl) was added into the solution, for 30 min at room temperature. The optical density was read at the wavelength 650 nm against blank using UV-VIS spectrophotometer (Shimadzu UV 1201) (1 cm light path).

4.3 Standard solution

The standard curve was plot using the bovine serum albumin (BSA) at 0, 20, 40, 60, 80 and 100 mg%. The slope of curve was used to calculate the concentrations of protein in jejunal mucosa and liver.

5 Determination of mucosal disaccharidase activities in jejunum

5.1 Tissue preparation

Mucosal scrapping from the jejunum of broiler chicken were used as source of enzyme. Mucosal jejunal scrapping were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four part of distilled water weight by volume (w/v). Then the samples were centrifuged at 3,000 rpm (Centrifuge, GLC-2B, SORVALL), for 10 min and remove the supernatant for disaccharidase determination.

5.2 Assay procedure

An aliquot of sample was diluted with distilled water (maltase 1: 1,000, sucrase 1: 100). The test tube containing 100 µl of the diluted enzyme solution were place in a water bath for 5 min. Then 10 µl of the substrate buffer solution were added (maltose or sucrose). Then incubated in 37 ° C for 60 min, 2000 µl of enzyme reagent (Glucose liquicolor, Human Gesellschaft fur Biochemica and Diagnostica mbH, Germany) was added and mixed. These test tubes were place in the water bath at 37 ° C for 5 min. The optical density was read at wavelength 500 nm against reagent blank, by using UV-VIS spectrophotometer (Shimadzu UV 1201; 1 cm light path).

5.3 Standard solution

The standard curve was plot using the glucose at 10, 20, 30, 40 and 50 mg%. The slope of the curve was used for calculated the concentration of glucose.

Results were expressed as specific activity (unit per milligram brush border protein). One unit was defined as disaccharidase that hydrolyzed 1 µmol of the substrate per min under the experimental condition.

The disaccharidase activity was obtained by the following formula:

$$\frac{a \times b}{n \times 1080} \quad \text{units/ml}$$

where

a	=	µg glucose liberated in 60 minutes
b	=	dilution factor for enzyme solution
n	=	number of glucose molecules per molecule of disaccharide (for maltase, n=2 and sucrase n=1)

6 Assay of glutathione peroxidase activities.

6.1 Tissue preparation

Liver samples were washed in potassium phosphate buffer (50 mM, pH 7.0) and then homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) in a nine volume of same buffer. The liver homogenate was centrifuged (3,500 rpm for 30 min) and the enzyme activities in the resulting supernatant were determined. GSH-Px was determined by using spectrophotometrically (Shimadzu UV 1201; 1 cm light path).

6.2 Assay procedure

A 170 μl of supernatant was incubated with start reagent (1 part of 8.4 mM NADPH in 1% NaHCO$_3$ and 2 parts of 2.2 mM H$_2$O$_2$ in distilled water). Then added working GSSG-R and GSH (26 parts of 5.8 mM GSH in 50 mM phosphate buffer and 1 part of 10 U/ml GSSG-R in 50 mM phosphate buffer and EDTA and sodium azide) in a final volume of 3.06 ml. The absorbance was read at wavelength 340 nm, as the rate of disappearance of NADPH using UV-VIS spectrophotometer. Reading was made against reagent blank. The change in A$_{340}$ due to NADPH oxidation is monitored and is indicative of GSH-PX activity. The over-all 2- steps reaction is:

$$R\text{-}O\text{-}O\text{-}H + 2GSH \xrightarrow{\text{GSH-Px}} R\text{-}O\text{-}H + H_2O + GSSG \xrightarrow{\text{GSSG-R}} 2GSH$$

NADPH   NADP+

Where: R-O-O-H; H$_2$O$_2$ or organic peroxide, 2GSH; reduced glutathione, R-O-H; hydroxyl radical, H$_2$O; water, GSSG; oxidized glutathione, GSSG-R; glutathione reductase, NADPH; reduced Nicotinamide Adenine Dinucleotide Phosphate , NADP; Nicotinamide Adenine Dinucleotide Phosphate.

One unit of GSH-Px activity was defined as the amount of GSH-Px that transforms 1 nmol NADPH to NADP per min in experimental condition. The specific activity was expressed in milliunit per milligram of liver protein.

The GSH-Px activity was obtained by the following formula:

GSH-Px activity (mU/mg protein)   =

$((\Delta A_{340}/min/extinction\ coefficient) \times D \times d \times (TV/SV)) \times 1/SW$

Where:

Extinction coefficient for NADPH is 0.00622 μM^{-1} cm^{-1} at 340 nm.

D   =   Dilution factor

d   =   light path in cm

TV   =   total volume

SV   =   sample volume

SW   =   sample weight

7 Determination of liver catalase activity

7.1 Tissue preparation

Liver samples were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four parts of detergent (1% Triton X-100) weight by volume (w/v).

7.2 Assay procedure

The test tube containing 100 μl of stock homogenate and 1.9 ml of phosphate buffer were mixed. Then 1 ml of 30 mM H_2O_2 was added to start the reaction. The decrease optical density was read at wavelength 240 nm every 30 sec for 1-2 min using UV-VIS spectrophotometer (Shimadzu UV 1201; 1 cm light path). Reading was made against reagent blank consisting of 2 ml of buffer and 1 ml of hydrogen peroxide.

7.3 Standard solution

Standard curve was plotted using catalase bovine liver at 20, 60, 100, 200 and 300 U/100 μl. The slope the curve was used to calculate the concentration of catalase.

One unit of CAT activity was defined as 1 μmol of hydrogen peroxide decomposed per min. The specific activity was expressed in unit per milligram of liver protein.

The CAT activity was obtained by the following formula:

$$\text{CAT activity (U/mg protein)} = (\Delta A_{240}/min \times S \times D) \times 1/SW$$

Where:

S = slope of CAT standard curve

D = dilution factor

SW = sample weight

8 Determination of superoxide dismutase activity

8.1 Tissue preparation

Liver samples were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four parts of phosphate buffer, weight by volume (w/v). The liver homogenate was centrifuged (3,500 rpm for 30 min) and the enzyme activities in the resulting supernatant were determined.

8.2 Assay procedure

The test tube containing 2.6 ml of phosphate buffer (10 mM, pH 7.4), 100 μl of cytochrome C (0.3mM), 100 μl of xanthine (1.5 mM), 100 μl of xanthine oxidase solution (0.2 unit/ml) and 100 μl of supernatant, respectively. Then the test tubes were placed in a water bath at 37 ° C for 20 min. The optical density was read at wavelength 550 nm, using UV-VIS spectrophotometer (Shimadzu UV 1201) (1 cm light path). The increase absorbance was recorded every 30 sec for 1-2 min. Reading was made against the blank.

8.3 Standard solution

Standard curve was plotted using superoxide dismutase from bovine erythrocytes at 10, 30, 60, 120 and 240 U/100 μl. The slope the curve was used for calculate the concentration of superoxide dismutase.

Unit of SOD activity was defined by the amount of the enzyme required to inhibit the rate of reduction of cytochrome C by 50 % in coupled system with xanthine and xanthine oxidase. The specific activity was expressed in unit per milligram of liver protein. The SOD activity was obtained by the following formula:

$$\text{SOD activity (U/mg protein)} = (\Delta A_{550}/\text{min} \times S \times D) \times 1/SW$$

Where:

S	=	slope of SOD standard curve
D	=	dilution factor
SW	=	sample weight

9 Calculation of the growth performance

The feed intake of chicks was recorded weekly. Body weight as pen basis was measured for growth performance calculation on days 1, 14, 28 and 42. Individual body weight was measured from 50% of chicks in each pen for body weight uniformity calculation on days 14 and 28, and 100% of chicks on day 42. Mortality and culled chicks were recorded daily.

Body weight gain (g/b) = Final body weight – Initial body weight

Average daily gain (ADG, g/b/d) = Body weight gain / Days

Feed intake (g/b) = Total feed intake / Final chicks no.

Daily feed intake (DFI, g/b/d) = Feed intake / Days

Mortality rate (%) = (No. of dead chicks / Total chicks no.) x100

Culled rate (%) = (No. of culled chicks / Total chicks no.) x100

Flock uniformity (%) = 100 – (%CV)

Feed conversion ratio (FCR, kg feed/kg body weight gain) =

Total pen feed / ((Final pen W + culled and dead W) - Initial pen W)

10 Statistical analysis

All data were presented as individual mean in each treatment and pooled SEM. The effects of treatment were analyzed using One - Way Analysis of Variance (ANOVA). The data show main effects (feeding regimens, Se supplementation) and interaction between both main effects. Individual mean was compared with Duncan's Multiple Range test by Stat - graphic program. Significant level was set at p<0.05.

CHAPTER IV

RESULTS

Effect on growth performance

Growth performance of starter period (days1-14 of age) are shown in Table 4. Chicks in treatments 1-3 were given their diets immediately after the allocation finished, while chicks in treatments 4-6 were fasted for 48 h after allocation finished. The results showed that fasted chicks for 48 h had significantly ($p<0.05$) lower body weight gain, average daily gain (ADG), feed intake and body weight uniformity (Figures 16, 18, 20 and 26, respectively). On the contrary, there were no significant difference on growth parameter from the effect of selenium supplementation (Figures 17, 19, 21, 23, 25 and 27, respectively). In addition, interaction was between two study factors (feeding regimen and selenium supplementation) on the body weight gain and ADG. The body weight gain and ADG of fed chicks that received organic selenium (OS) were markedly ($p<0.001$) greater than fed chicks received nil (treatment 1) or fasted chicks (treatments 4-6). The final body weight, body weight gain and ADG of all fed chicks (treatments 1-3) were markedly ($p<0.001$) higher than fasted birds (treatments 4-6) (Figure 6 and 7, respectively) and there was no significant difference on body weight gain and ADG of chicks in groups 4-6. The feed intake (g/b) and DFI (g/b/d) of chicks in treatments 1-3 were also higher ($p<0.001$) than those fasted chicks (Figure 8 and 9, respectively). The effect of selenium on feed conversion ratio (FCR) was very promising. Fed chicks received diets supplemented with selenium in both forms (OS and IOS) had better FCR ($p<0.05$) than fed control (Figure 10). The body weight uniformity of fed chicks was much better than fasted chicks ($p<0.05$). Fed chicks that received OS (treatment 2) had significantly ($p<0.05$) greater body weight uniformity than control groups (Figure 11). There were no significant difference on mortality rate.

Table 4 Effect of posthatch feeding or fasting on growth performance of broilers fed practical diet with supplemented organic or inorganic selenium (Days 1-14).

Group	Feeding regimen	Se supplementation (ppm)	Initial body wt. (g/b)	Final body wt. (g/b)	Body wt. gain (g/b)	ADG (g/b/d)	Feed intake (g/b)	DFI (g/b/d)	Feed conversion ratio	Mortality (%)	Culled* (%)	Body wt. uniformity (%)
	Treatment											
1	Fed	nil	43.1	419.8[b]	376.7[b]	26.90[b]	474.9[a]	33.92[a]	1.261[a]	0.31	5.31	90.34[ab]
2	Fed	0.2 as OS (Sel-Plex)	43.0	435.9[a]	392.9[a]	28.07[a]	475.7[a]	33.98[a]	1.211[b]	1.25	5.00	91.63[a]
3	Fed	0.2 as IOS (Na_2SeO_3)	43.0	427.8[ab]	384.8[ab]	27.48[ab]	465.4[a]	33.11[a]	1.210[b]	0.94	5.00	89.78[abc]
4	Fasted	nil	42.9	333.4[c]	290.5[c]	20.75[c]	340.7[b]	28.39[b]	1.173[b]	0.00	5.31	88.09[bc]
5	Fasted	0.2 as OS (Sel-Plex)	43.1	328.7[c]	285.7[c]	20.39[c]	340.4[b]	28.37[b]	1.192[b]	0.94	5.00	89.57[abc]
6	Fasted	0.2 as IOS (Na_2SeO_3)	42.9	335.3[c]	292.4[c]	20.87[c]	352.0[b]	29.33[b]	1.204[b]	0.63	5.00	87.45[c]
Pooled SEM			0.092	3.82	3.82	0.27	5.46	0.37	0.02	0.16	0.04	0.82
CV, %			0.60	2.84	3.21	0.030	3.78	0.03	3.98	36.37	4.15	0.02

Source	df											
			----- p-value -----									
Treatment diet	5		0.349	0.001	0.001	0.001	0.001	0.001	0.003	0.378	0.572	0.013
Feeding regimen (A)	1		0.284	0.001	0.001	0.001	0.001	0.001	0.017	0.474	1.000	0.009
Se supplementation (B)	2		0.505	0.298	0.301	0.301	0.985	0.985	0.679	0.171	0.210	0.111
(A) x (B)	2		0.562	0.044	0.042	0.038	0.113	0.077	0.064	0.991	1.000	0.988

Main effect

Feeding regimen

Fed			43.03	427.83[a]	384.80[a]	27.48[a]	472.03[a]	33.67[a]	1.227[a]	0.83	5.10	90.58[a]
Fasted 48 h			42.95	332.48[b]	289.53[b]	20.67[b]	344.38[b]	28.7[b]	1.190[b]	0.52	5.10	88.37[b]

Se supplementation

nil			42.98	376.59	333.61	23.82	407.8	31.15	1.217	0.16	5.31	89.21
0.2 ppm as OS (Sel-Plex)			43.05	382.33	339.27	24.23	408.08	31.17	1.202	1.09	5.00	90.60
0.2 ppm as IOS (Na_2SeO_3)			42.94	381.54	338.60	24.17	408.73	31.22	1.207	0.78	5.00	88.61

[a,b,c] Means within column for treatment diet or for each main effect with no common superscript differ significantly ($p < 0.05$)

* For tissue sample collection and culling.
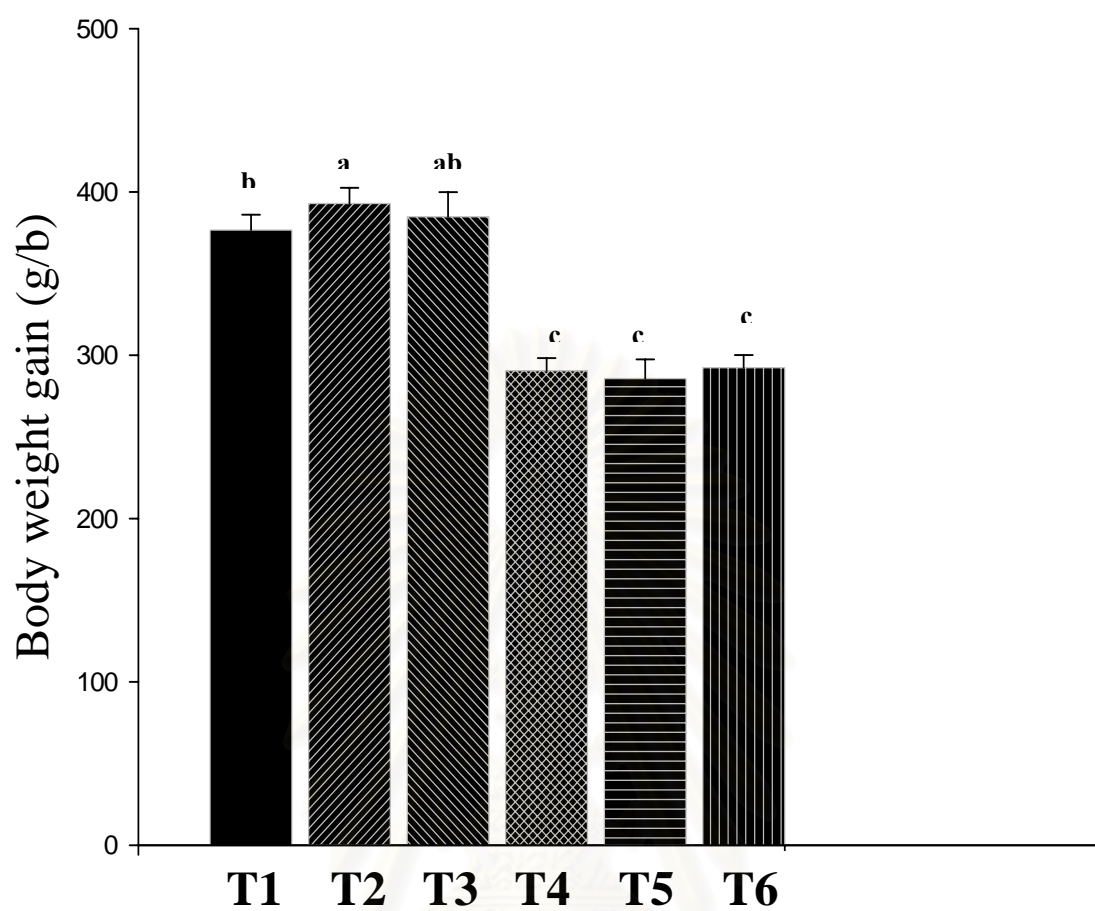
Figure 6 Effect of posthatch feeding or fasting on body weight gain (g/b) of broilers

fed on practical diet with supplemented organic or inorganic selenium (days 1-14).
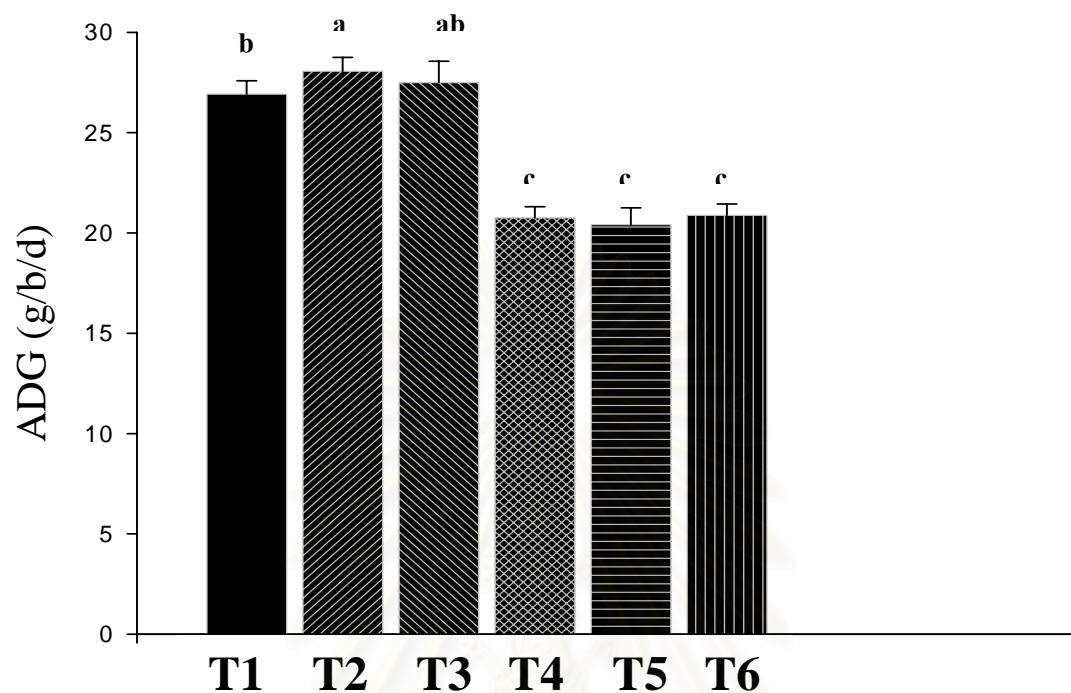
a,b,c Different superscripts mean significantly different (p<0.05)

Figure 7 Effect of posthatch feeding or fasting on average daily gain (g/b/d) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 1-14).
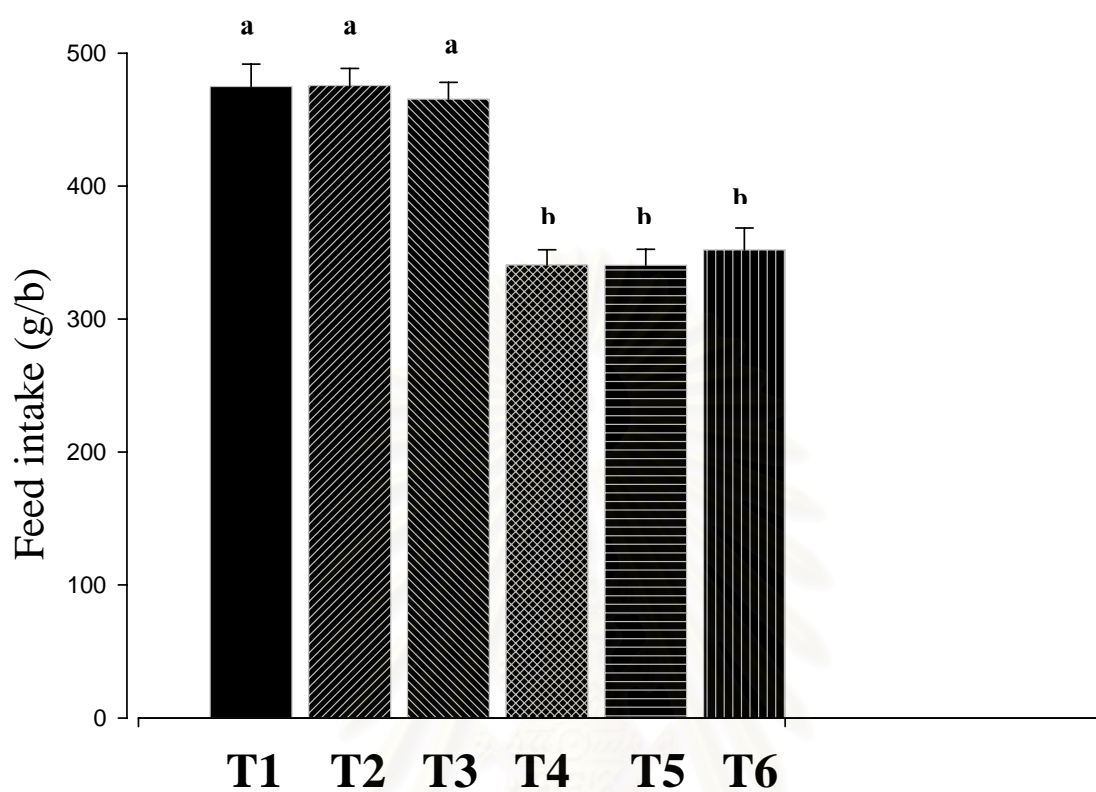
<superscript>a,b</superscript> Different superscripts mean significantly different (p<0.05)

Figure 8 Effect of posthatch feeding or fasting on feed intake (g/b) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 1-14).
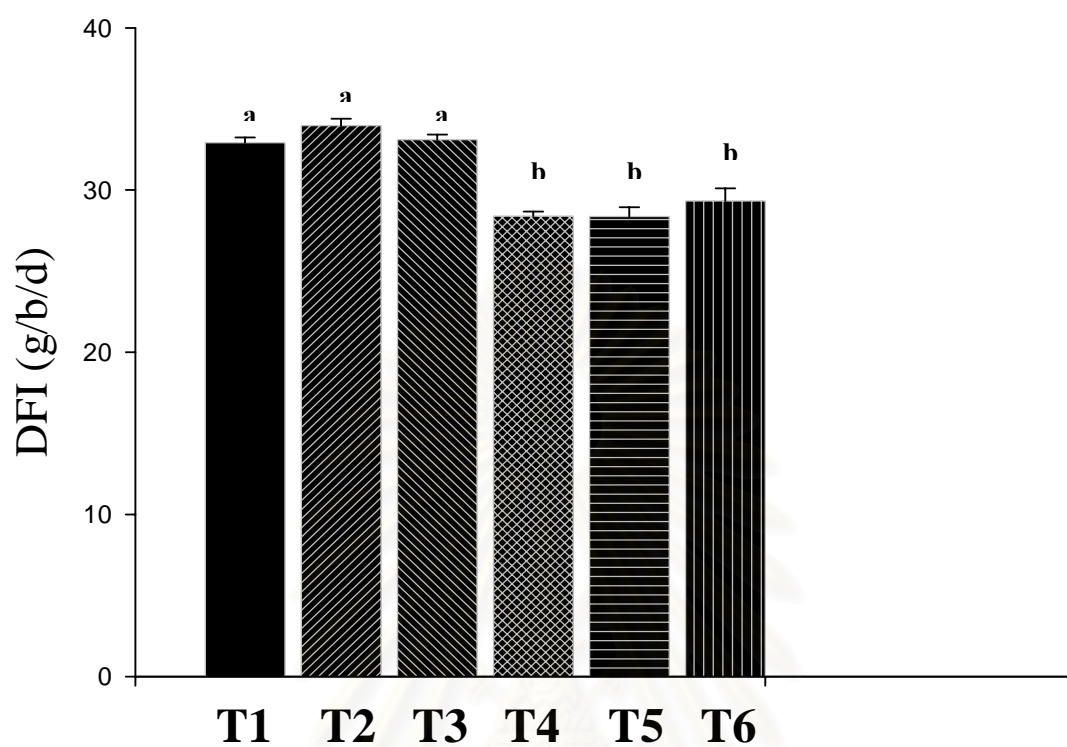
a,b Different superscripts mean significantly different (p<0.05)

Figure 9 Effect of posthatch feeding or fasting on daily feed intake (g/b/d) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 1-14).
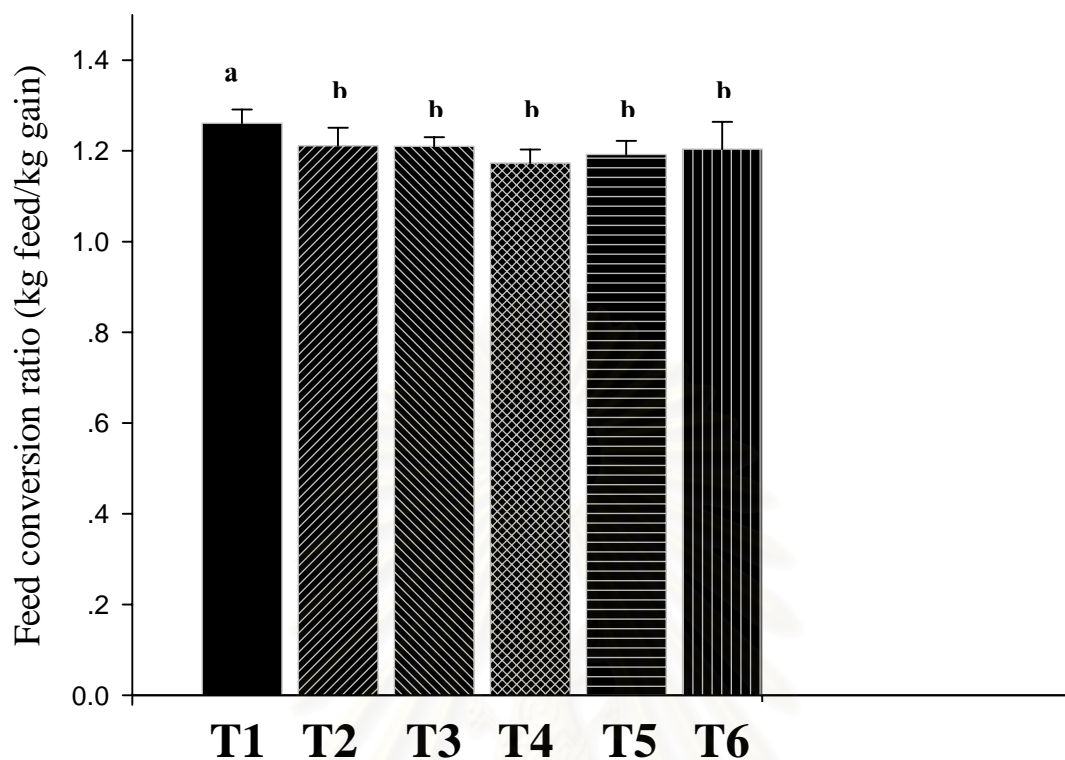
a,b Different superscripts mean significantly different (p<0.05)

Figure 10 Effect of posthatch feeding or fasting on feed conversion ratio (kg feed/kg gain) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 1-14).
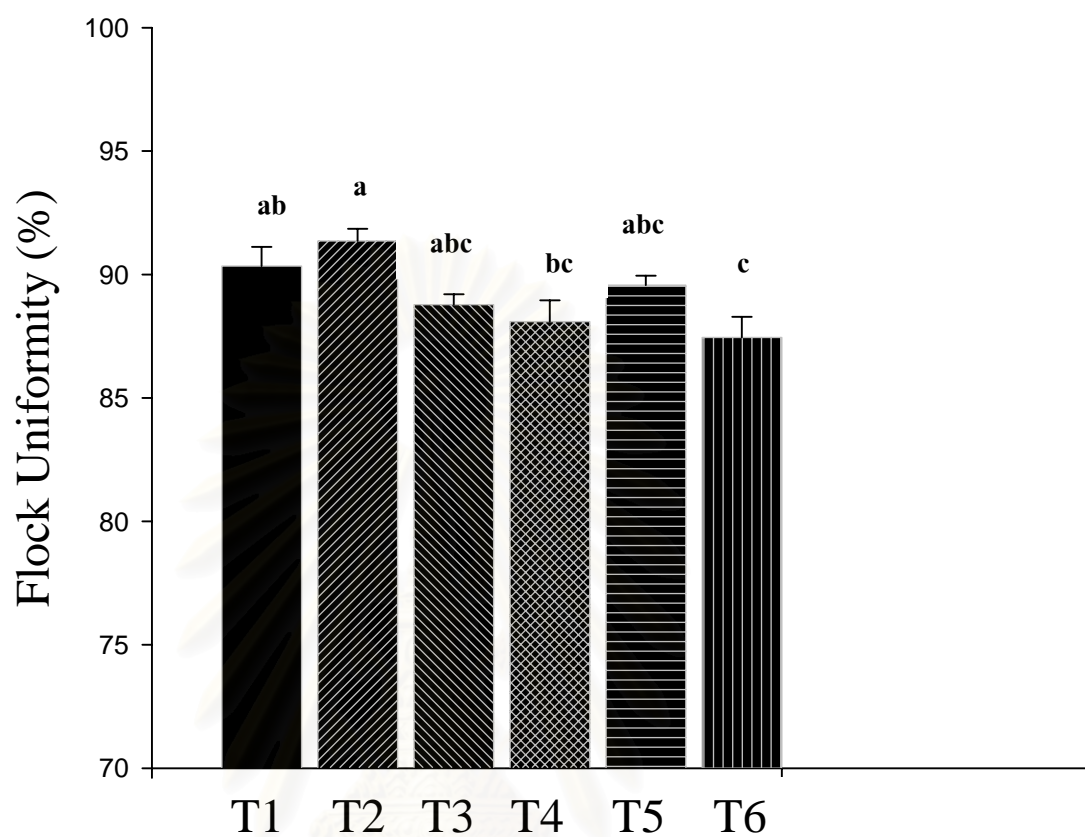
a,b,c Different superscripts mean significantly difference (p<0.05)

Figure 11 Effect of posthatch feeding or fasting on flock uniformity (%) of broiler fed on practical diet supplemented with organic or inorganic selenium (days 1-14).

Growth performance at grower period (days 15-28 of age), are shown in Table 5. It was found that the final body weight of fed chicks were still greater than fasted chicks ($p < 0.05$), but there were no significant difference on body weight gain and ADG. In addition, fed chicks had significantly ($p < 0.05$) higher feed intake (116.80 g/b) and DFI (8.32 g/b/d) than fasted chicks (Figures 12 and 13, respectively). On the contrary, fasted chicks for 48 h had significantly ($p < 0.001$) better FCR than fed chicks (Figure 22). Effect of selenium supplementation (main effect), chicks that received diet supplemented with OS (treatments 2, 5) had significantly ($p < 0.001$) better FCR than other groups (Figure 23). It was found that treatment diets affected FCR. Fasted chicks that received OS and nil had significantly ($p < 0.001$) better FCR than other groups (Figure 14). Moreover, OS supplemented groups had significantly ($p < 0.05$) higher body weight uniformity than inorganic selenium (IOS) supplemented groups and control groups. Furthermore, it was found that there were an effect from treatment diet. Fasted chicks that received OS had significantly ($p < 0.05$) higher body weight uniformity than fasted chicks that received IOS and nil (Figure 15). There were no significant difference on mortality rate. Control group had slightly higher ($p > 0.05$) mortality rate than selenium supplemented groups.

At finisher period (days 29-42 of age), growth performance are shown in Table 6. It was demonstrated that the final body weight of fed chicks were still greater than fasted chicks ($p < 0.05$), but there were no significant difference on body weight gain and ADG. In addition, fed chicks had significantly ($p < 0.05$) higher DFI than fasted chicks (2.4 g/b/d). In contrast, fasted chicks had significantly ($p < 0.05$) better FCR than fed chicks. Moreover, body weight uniformity of fed chicks were significantly ($p < 0.05$) higher than fasted chicks and OS supplemented group and control group had significantly ($p < 0.05$) higher body weight uniformity than IOS supplemented group. There were no significant difference on mortality rate in this period, though fed chick had slightly ($p > 0.05$) higher mortality rate than fasted chicks (Figure 24). It was found that IOS supplemented group and control group had slightly ($p > 0.05$) higher mortality rate than OS supplemented group(Figure 25).

Table 5 Effect of posthatch feeding or fasting on growth performance of broilers fed practical diet with supplemented organic or inorganic selenium (Days 15-28).

| Group	Feeding regimen	Se supplementation (ppm)	Initial body wt. (g/b)	Final body wt. (g/b)	Body wt. gain (g/b)	ADG (g/b/d)	Feed intake (g/b)	DFI (g/b/d)	Feed conversion ratio	Mortality (%)	Culled* (%)	Body wt. uniformity (%)
1	Fed	nil	419.8[b]	1275.8[a]	856.1	61.1	1347.9[a]	96.27[a]	1.575[ab]	1.33	2.98	93.67[ab]
2	Fed	0.2 as OS (Sel-Plex)	435.9[a]	1328.0[a]	892.1	63.7	1368.8[a]	97.77[a]	1.535[ab]	0.33	2.67	94.78[a]
3	Fed	0.2 as IOS (Na$_2$SeO$_3$)	427.8[ab]	1290.2[a]	862.4	61.0	1360.7[a]	97.19[a]	1.588[a]	1.00	2.99	93.27[ab]
4	Fasted	nil	333.4[c]	1199.2[b]	865.8	61.8	1253.6[b]	89.54[b]	1.451[c]	0.67	2.64	92.29[b]
5	Fasted	0.2 as OS (Sel-Plex)	328.7[c]	1197.2[b]	868.5	62.0	1237.4[b]	88.38[b]	1.428[c]	1.32	3.32	94.49[ab]
6	Fasted	0.2 as IOS (Na$_2$SeO$_3$)	335.3[c]	1160.2[b]	825.0	58.9	1236.0[b]	88.35[b]	1.499[bc]	0.33	2.98	92.75[b]
Pooled SEM			3.82	16.14	13.90	1.26	15.17	1.26	0.01	0.20	0.07	0.61
CV, %			2.84	3.68	4.56	0.05	3.30	0.04	2.57	45.36	10.37	0.01

Source	df						p-value					
Treatment diet	5		*0.001*	*0.001*	*0.261*	*0.205*	*0.001*	*0.001*	*0.001*	*0.579*	*0.614*	*0.031*
Feeding regimen (A)	1		*0.001*	*0.001*	*0.154*	*0.210*	*0.001*	*0.001*	*0.001*	*0.820*	*0.708*	*0.172*
Se supplementation (B)	2		*0.298*	*0.095*	*0.059*	*0.025*	*0.951*	*0.960*	*0.001*	*0.808*	*0.825*	*0.028*
(A) x (B)	2		*0.044*	*0.194*	*0.253*	*0.318*	*0.449*	*0.450*	*0.460*	*0.374*	*0.331*	*0.659*

Main effect

Feeding regimen

Fed			427.83[a]	1298.0[a]	870.2	61.9	1359.1[a]	97.08[a]	1.566[a]	0.89	2.88	93.91
Fasted 48 h			332.48[b]	1185.5[b]	853.1	60.9	1242.3[b]	88.76[b]	1.459[b]	0.77	2.98	93.18

Se supplementation

nil			376.59	1237.5	861.0	61.49[ab]	1300.7	92.9	1.513[b]	1.00	2.81	92.98[c]
0.2 ppm as OS (Sel-Plex)			382.33	1262.6	880.3	62.87[a]	1303.1	93.47	1.482[c]	0.83	2.99	94.63[a]
0.2 ppm as IOS (Na$_2$SeO$_3$)			381.54	1225.1	843.7	59.94[b]	1298.3	92.77	1.544[a]	0.67	2.98	93.01[b]

[a,b,c] Means within column for treatment diet or for each main effect with no common superscript differ significantly (p < 0.05)
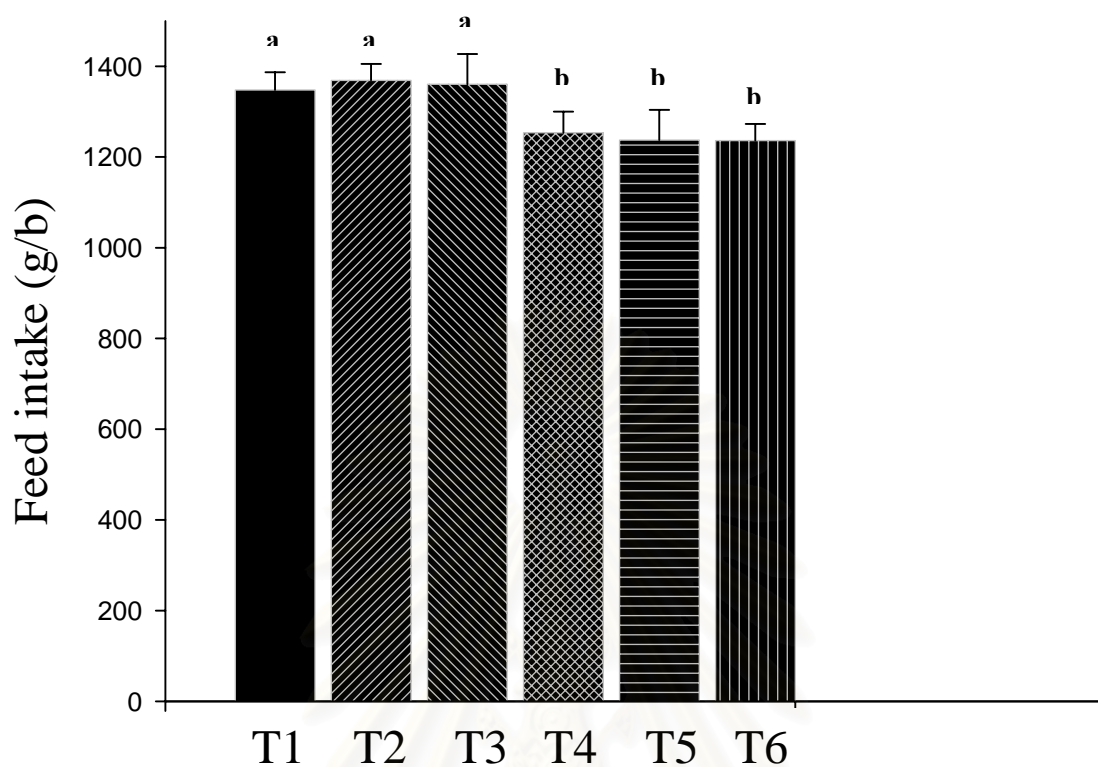
* For tissue sample collection and culling.

Table 6 Effect of posthatch feeding or fasting on growth performance of broilers fed practical diet with supplemented organic or inorganic selenium (Days 29-42).

	Treatment		Initial body wt.	Final body wt.	Body wt. gain	ADG	Feed intake	DFI	Feed conversion	Mortality	Culled*	Body wt. uniformity
Group	Feeding regimen	Se supplementation (ppm)	(g/b)	(g/b)	(g/b)	(g/b/d)	(g/b)	(g/b/d)	ratio	(%)	(%)	(%)
1	Fed	nil	1275.8[a]	2105.3[ab]	829.5	59.25	1808.4	129.2	2.192	4.53	3.47	90.73
2	Fed	0.2 as OS (Sel-Plex)	1328.0[a]	2225.5[a]	897.5	63.46	1900.8	135.8	2.133	2.38	3.43	91.84
3	Fed	0.2 as IOS (Na_2SeO_3)	1290.2[a]	2131.7[ab]	841.5	60.11	1830.3	130.7	2.193	2.06	3.46	89.78
4	Fasted	nil	1199.2[b]	2060.6[b]	861.4	61.53	1792.7	128.1	2.090	1.36	3.41	89.88
5	Fasted	0.2 as OS (Sel-Plex)	1197.2[b]	2084.2[b]	887.0	63.35	1836.0	131.1	2.080	0.70	3.49	89.53
6	Fasted	0.2 as IOS (Na_2SeO_3)	1160.2[b]	2025.5[b]	865.3	61.80	1760.3	125.7	2.042	2.04	3.43	88.85
Pooled SEM			16.14	40.47	34.89	2.49	43.99	3.42	0.05	0.25	0.01	0.74
CV, %			3.68	5.44	11.44	11.44	6.83	0.07	6.98	44.47	1.13	0.02
Source		*df*				*p-value*						
Treatment diet		5	*0.001*	*0.039*	*0.798*	*0.798*	*0.393*	*0.791*	*0.247*	*0.345*	*0.404*	*0.097*
Feeding regimen (A)		1	*0.001*	*0.008*	*0.508*	*0.508*	*0.147*	*0.038*	*0.004*	*0.074*	*0.681*	*0.045*
Se supplementation (B)		2	*0.095*	*0.129*	*0.300*	*0.301*	*0.178*	*0.506*	*0.613*	*0.453*	*0.855*	*0.038*
(A) x (B)		2	*0.194*	*0.453*	*0.789*	*0.788*	*0.746*	*0.776*	*0.404*	*0.432*	*0.295*	*0.875*

Main effect

Feeding regimen

Fed			1298.0[a]	2155.5[a]	855.0	61.10	1847.9	130.7[a]	2.172[a]	2.99	3.45	90.78[a]
Fasted 48 h			1185.5[b]	2056.8[b]	871.2	62.23	1796.3	128.3[b]	2.071[b]	1.36	3.44	89.42[b]

Se supplementation

nil			1237.5	2082.9	845.4	60.40	1800.5	128.6	2.141	2.95	3.44	90.30[ab]
0.2 ppm as OS (Sel-Plex)			1262.6	2156.8	890.5	63.60	1870.6	131.7	2.105	1.54	3.46	90.68[a]
0.2 ppm as IOS (Na_2SeO_3)			1225.1	2078.6	853.4	61.00	1795.3	128.2	2.118	2.05	3.44	89.32[b]

[a,b] Means within column for treatment diet or for each main effect with no common superscript differ significantly (p < 0.05)

* For tissue sample collection.

a,b Different superscripts mean significantly different (p<0.05)

Figure12 Effect of posthatch feeding or fasting on feed intake (g/b)of broilers fed on practical diet with supplemented organic or inorganic selenium (days 15-28).
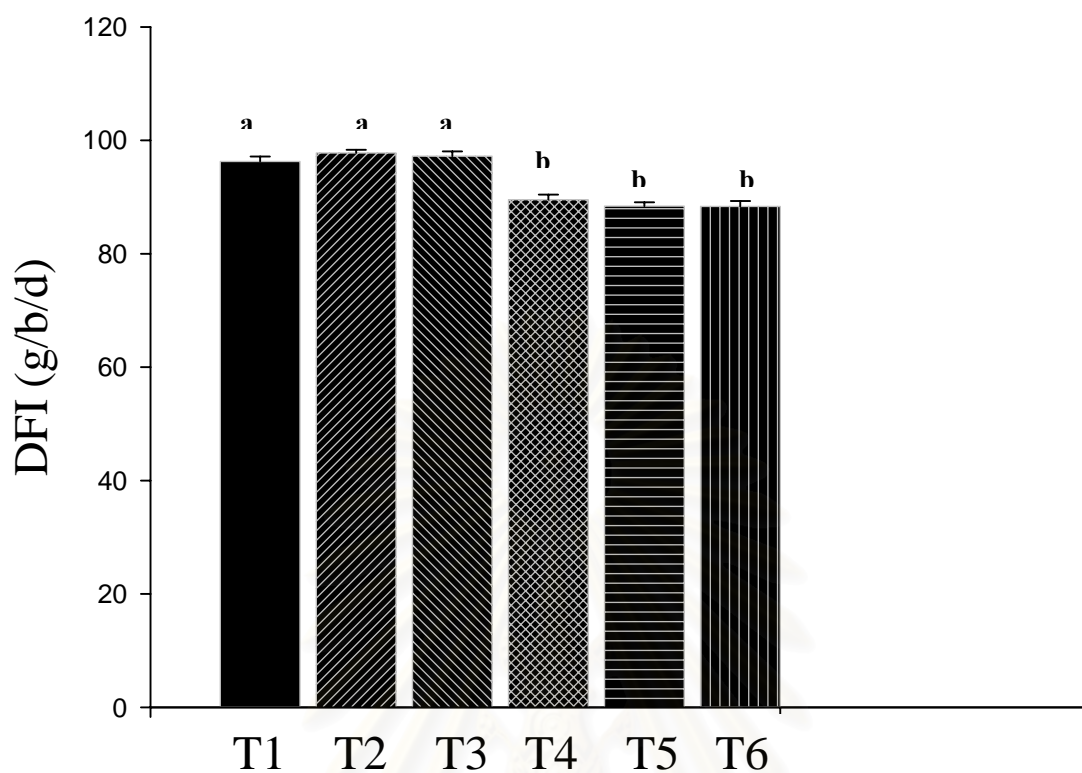
^{a,b} Different superscripts mean significantly different (p<0.05)

Figure 13 Effect of posthatch feeding or fasting on daily feed intake (g/b/d) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 15-28).
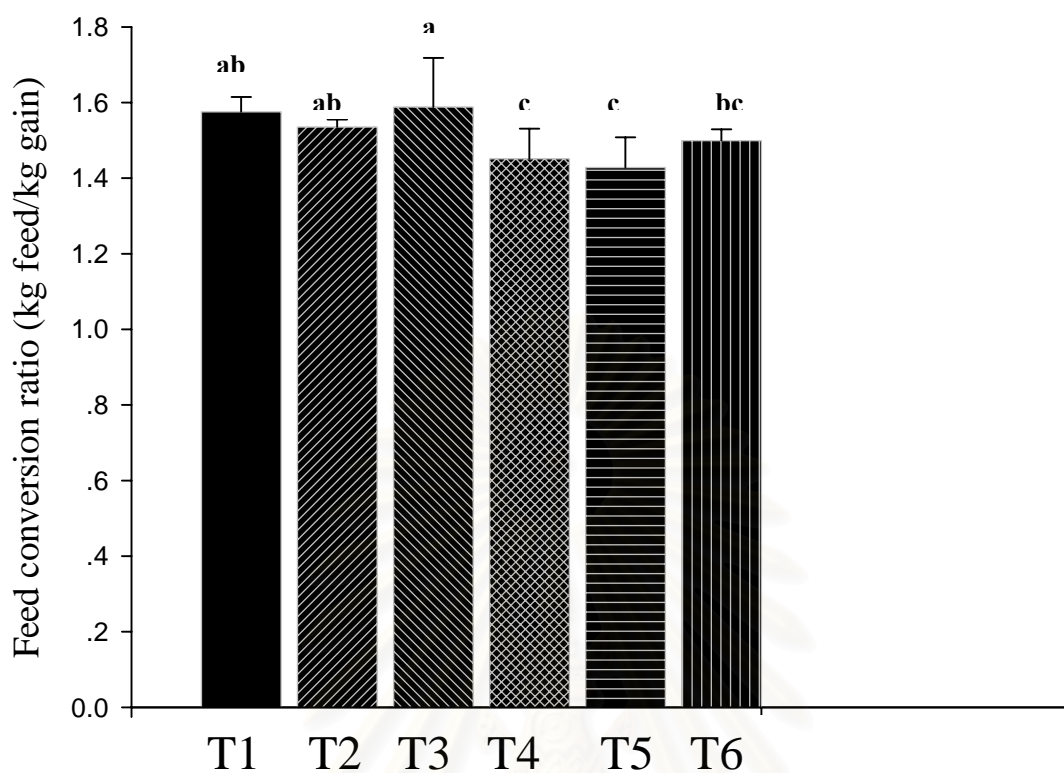
a,b,c Different superscripts mean significantly different (p<0.05)

Figure 14 Effect of posthatch feeding or fasting on feed conversion ratio (g/g) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 15-28).
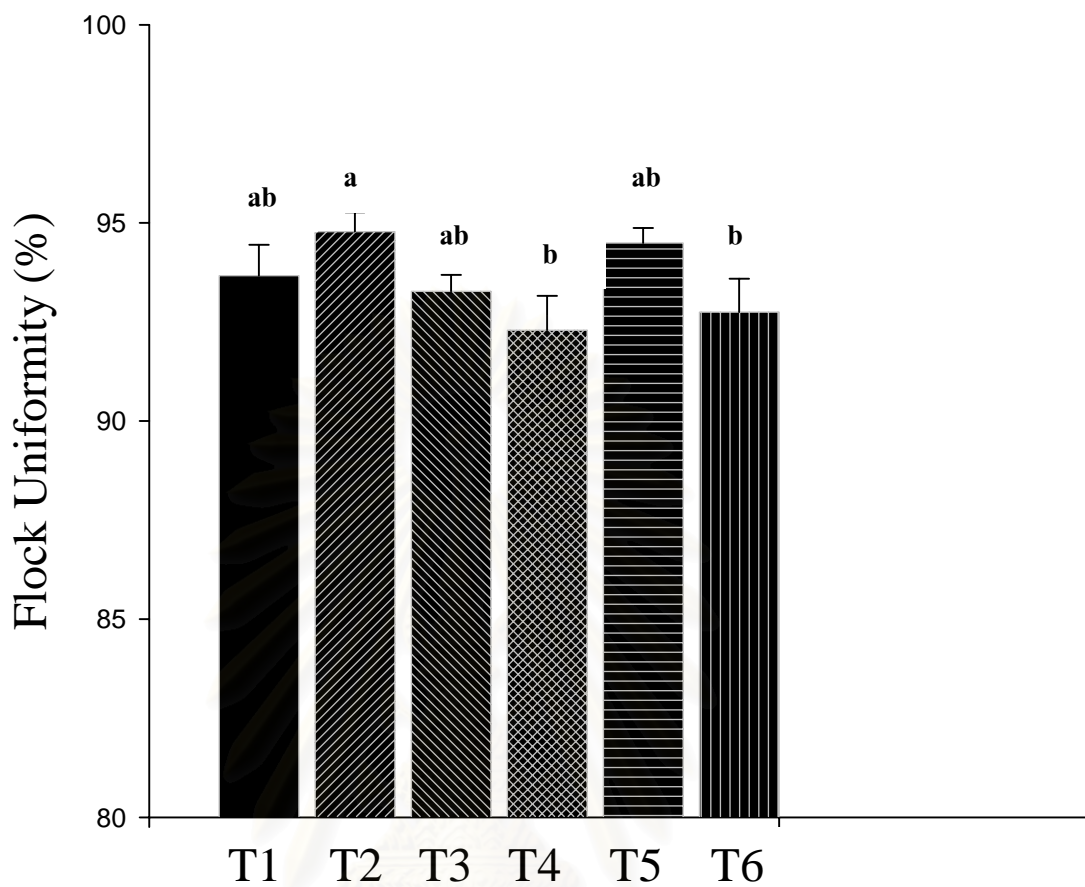
a,b,c Different superscript mean significantly difference (p<0.05)

Figure 15 Effect of posthatch feeding or fasting on flock uniformity (%) of broiler fed on practical diet supplemented with organic or inorganic selenium (days 15-28).
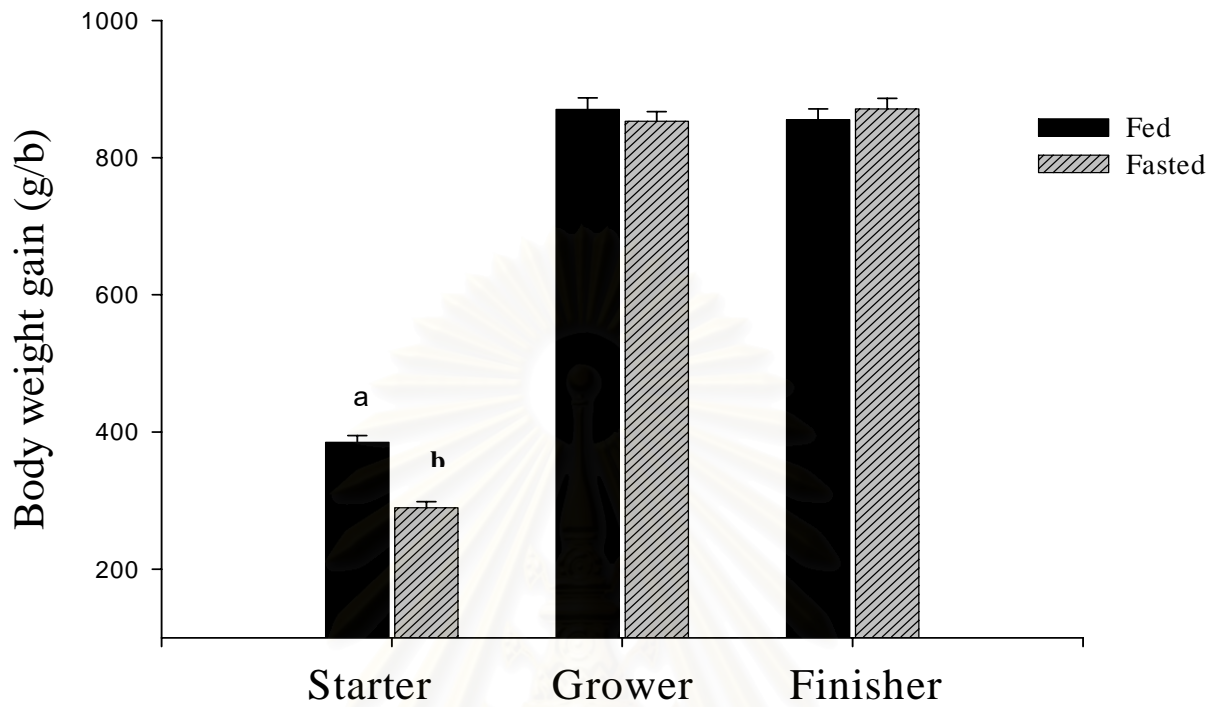
Figure 16 Effect of posthatch feeding or fasting on body weight gain (g/b) of broiler.

Figure 17 Effect of selenium supplementation on body weight gain (g/b) of broiler.

[a,b] Different superscript mean significantly difference in the same period (p<0.05)

Figure 18 Effect of posthatch feeding or fasting on average daily gain (g/b/d)of broiler.
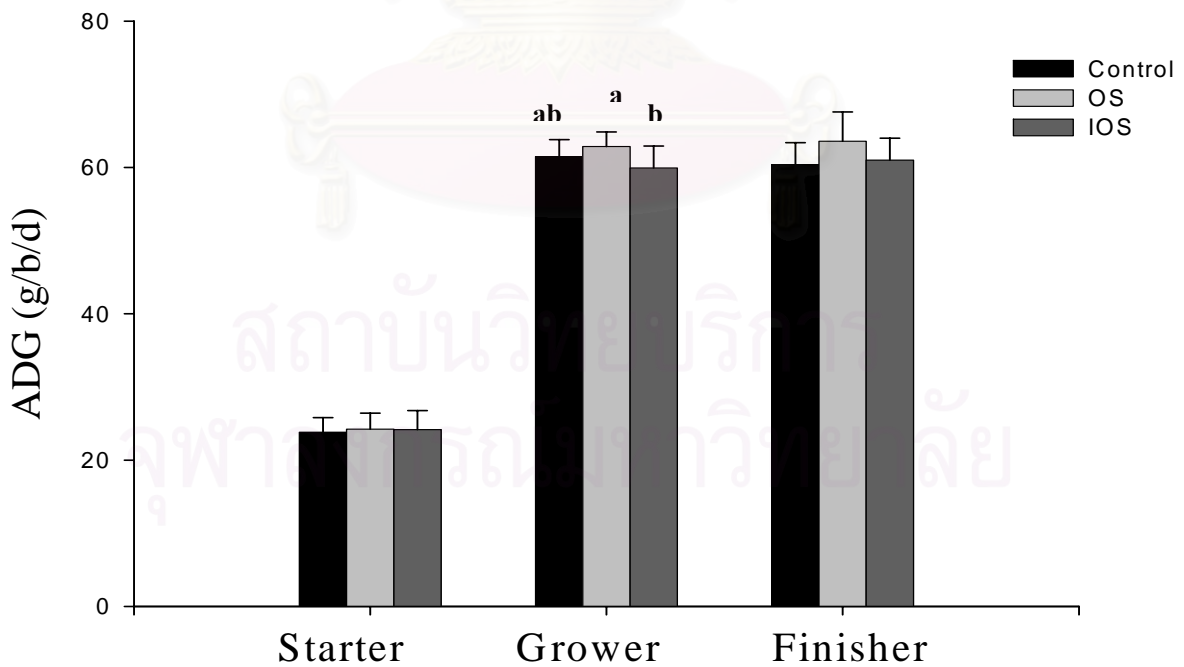
Figure 19 Effect of selenium supplementation on average daily gain (g/b/d) of broiler.

[a,b] Different superscript mean significantly difference in the same period (p<0.05)

Figure 20 Effect of posthatch feeding or fasting on feed intake (g/b) of broiler.
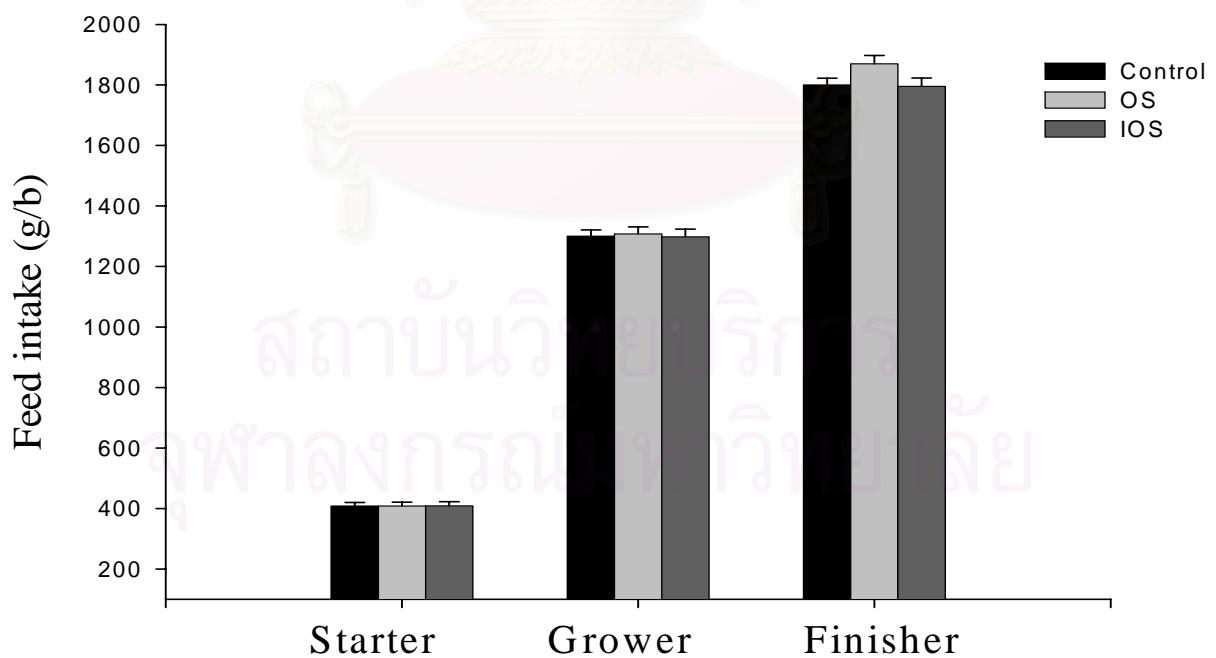
Figure 21 Effect of selenium supplementation on feed intake (g/b) of broiler.

[a,b] Different superscript mean significantly difference in the same period (p<0.05)
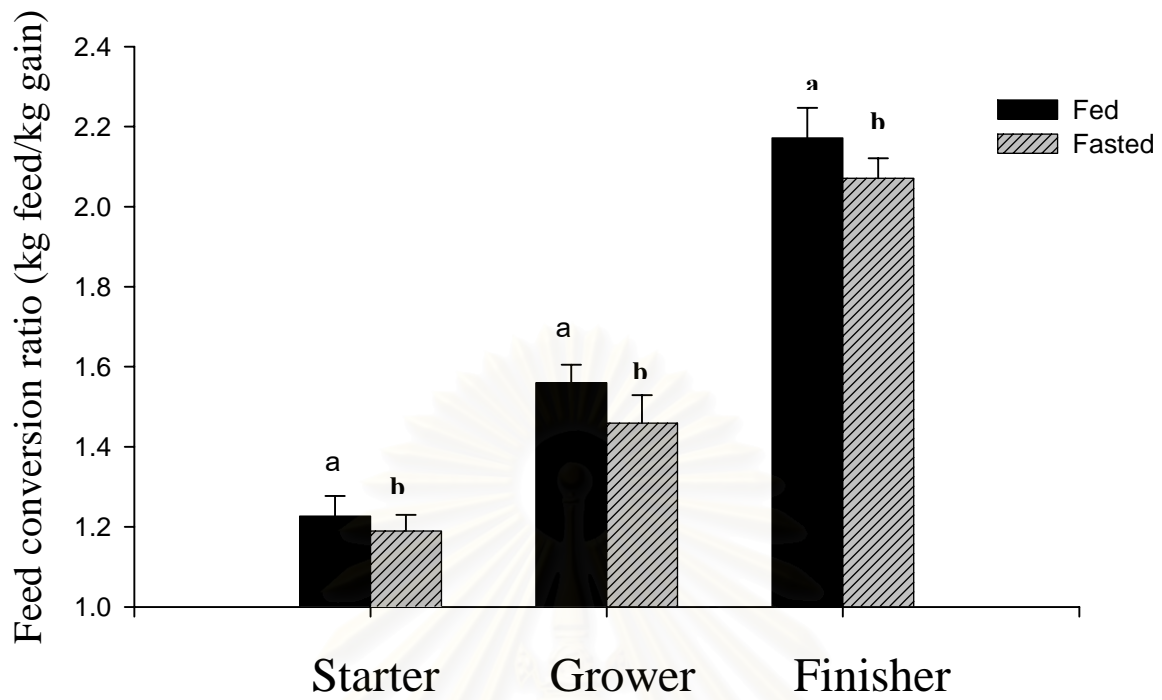
Figure 22 Effect of feeding or fasting on feed conversion ratio (kg feed/kg gain) of broiler.
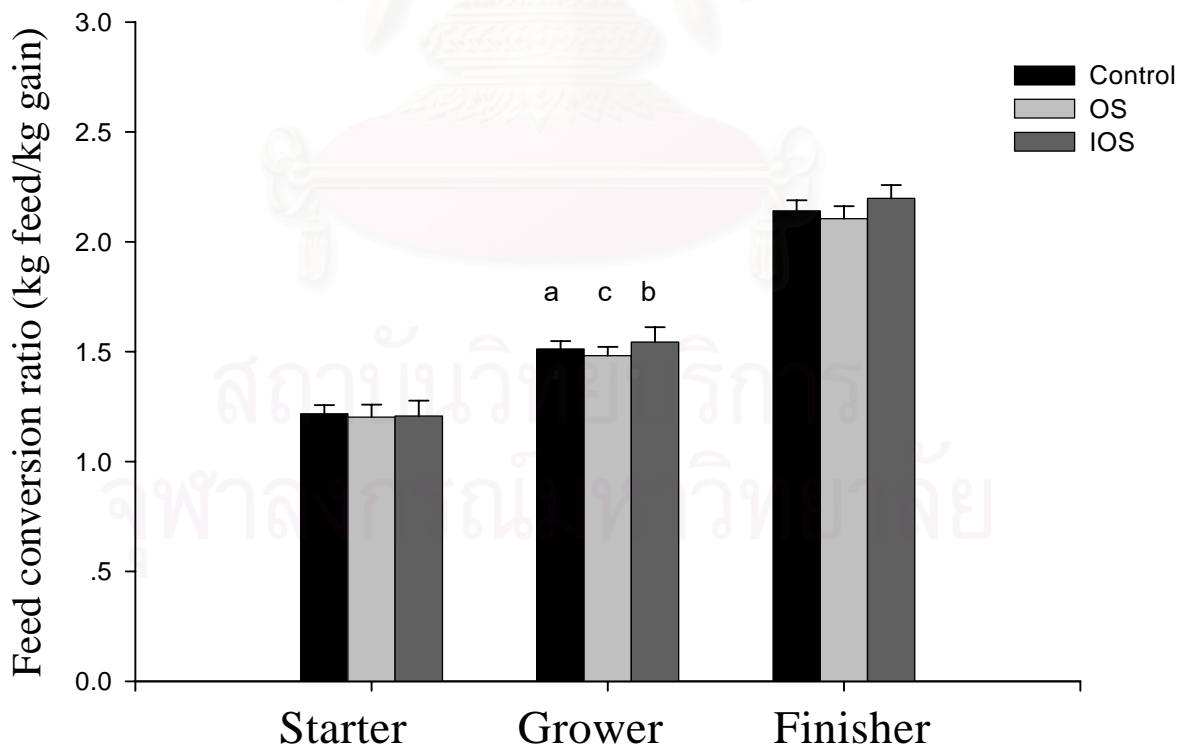
Figure 23 Effect of selenium supplementation on feed conversion ratio (kg feed/kg gain) of broiler.

[a,b,c] Different superscript mean significantly difference in the same period (p<0.05)
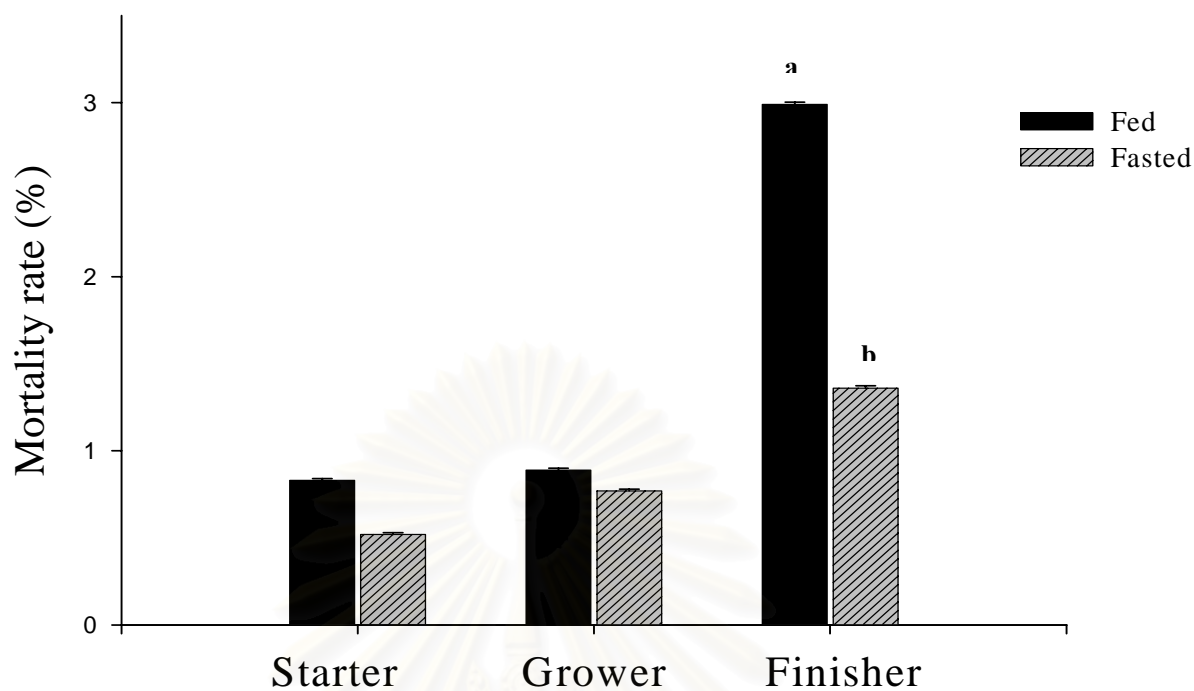
Figure 24 Effect of posthatch feeding or fasting on mortality rate (%) of broiler.
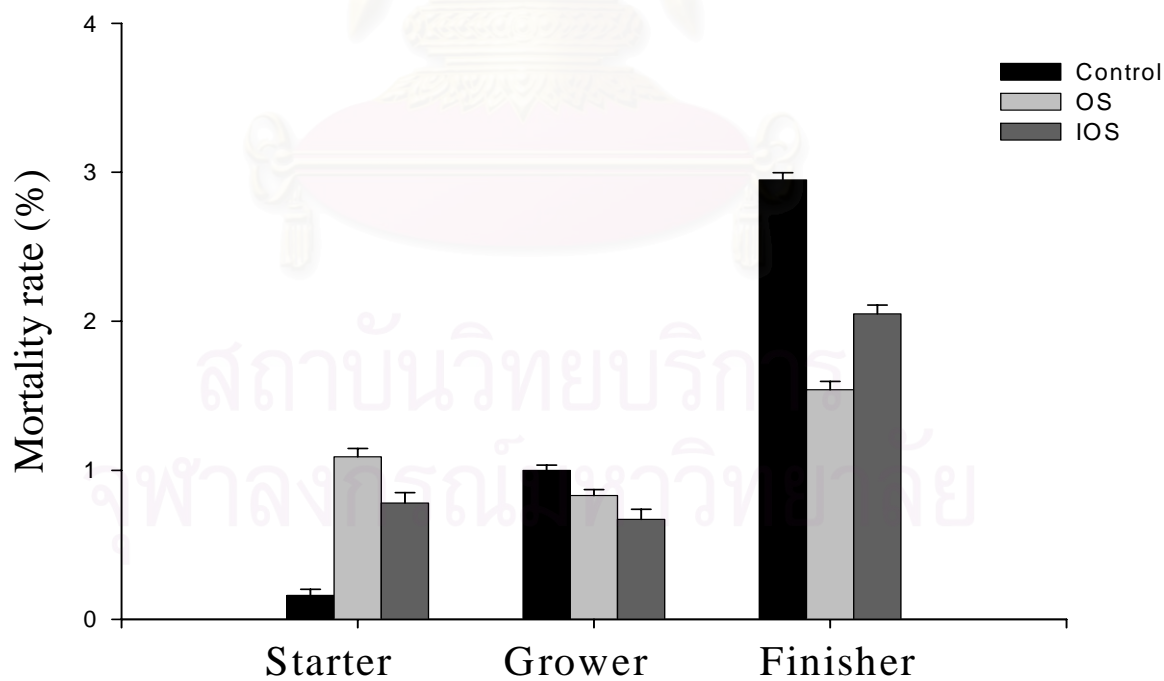
Figure 25 Effect of selenium supplementation on mortality rate (%) of broiler.

[a,b] Different superscript mean significantly difference in the same period (p<0.05)
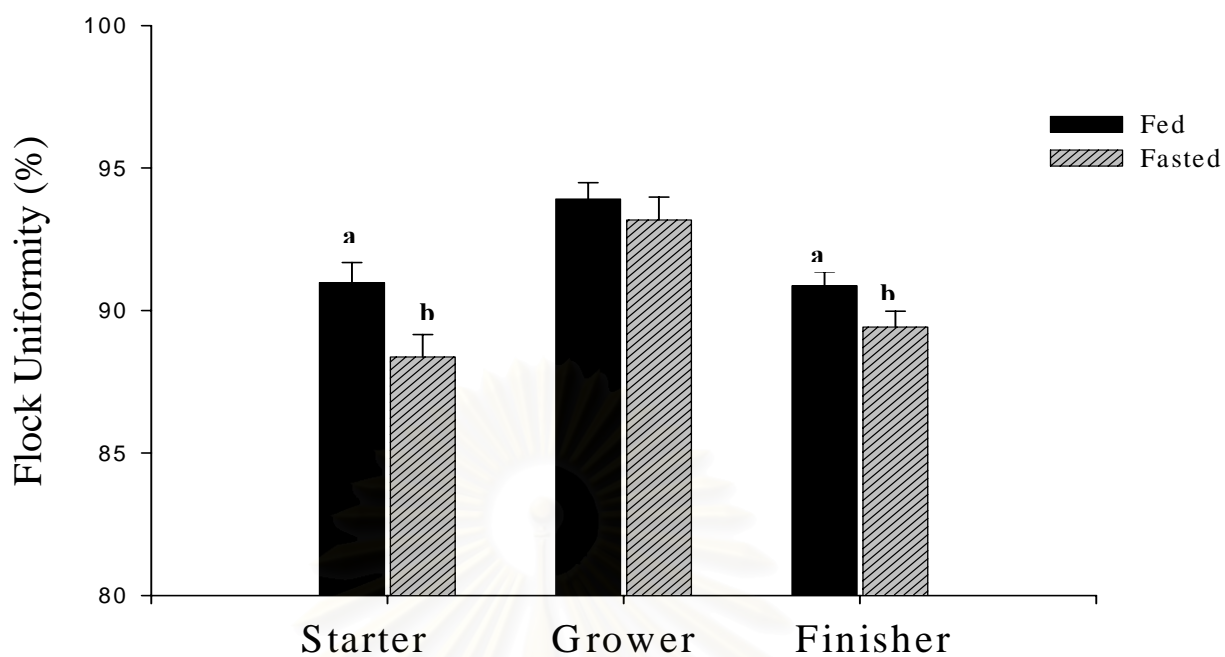
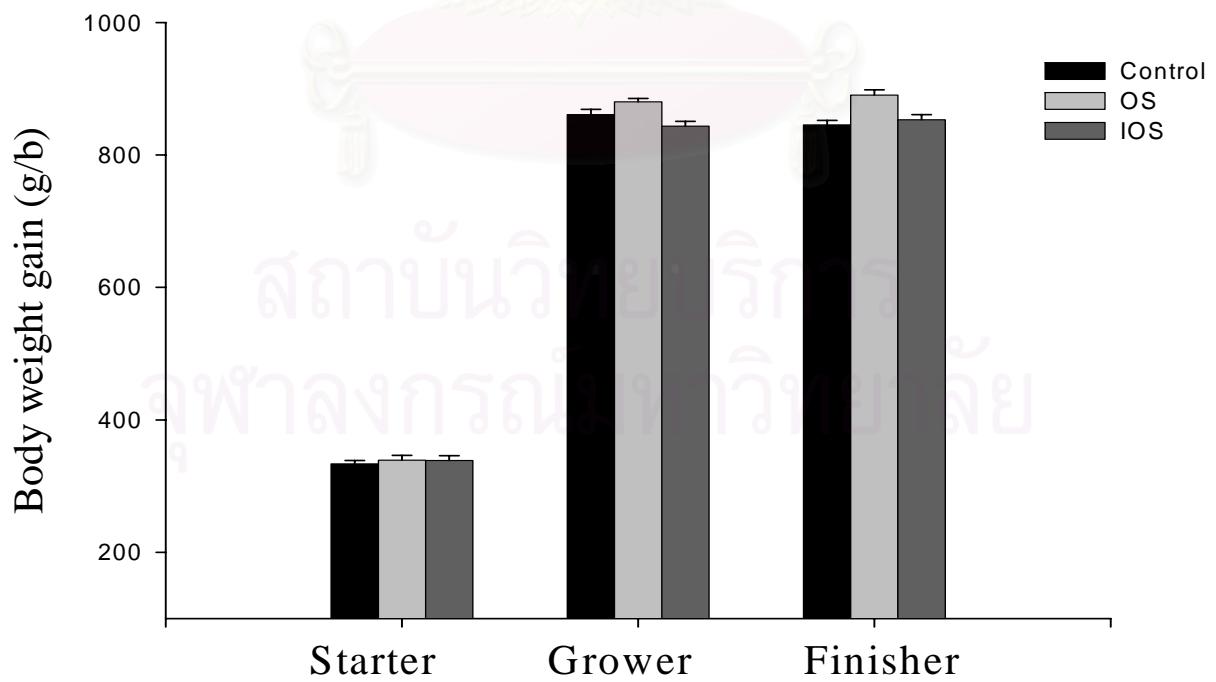**Figure 26** Effect of posthatch feeding or fasting on flock uniformity (%) of broiler.
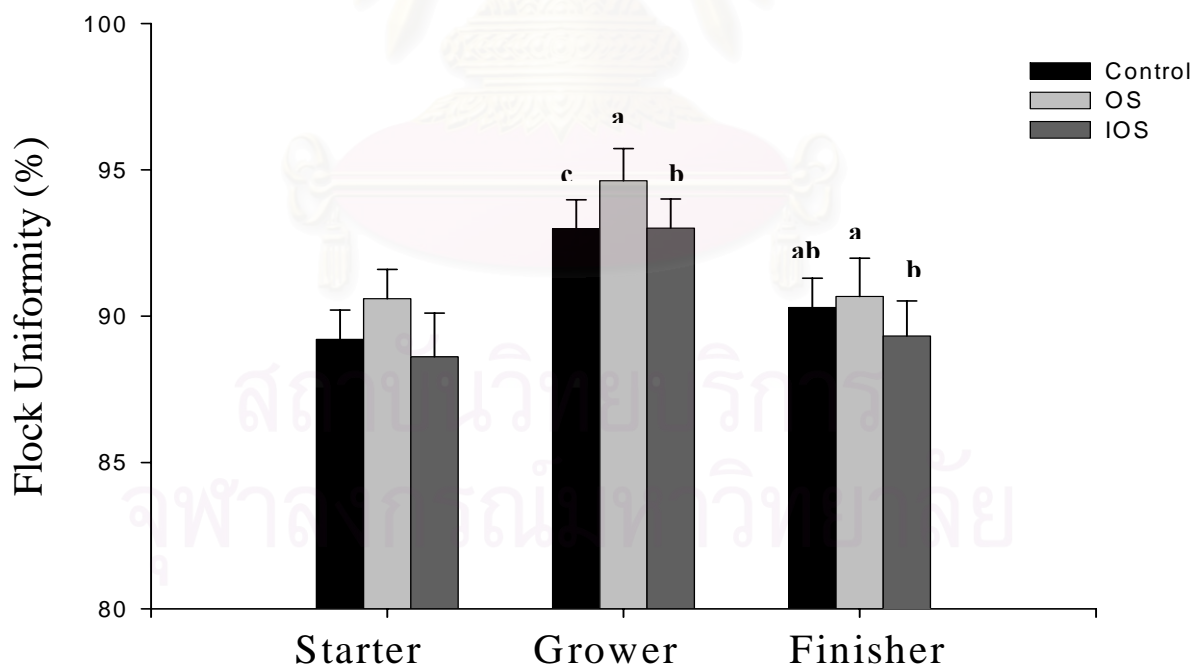
Figure 27 Effect of selenium supplementation on flock uniformity (%) of broiler.

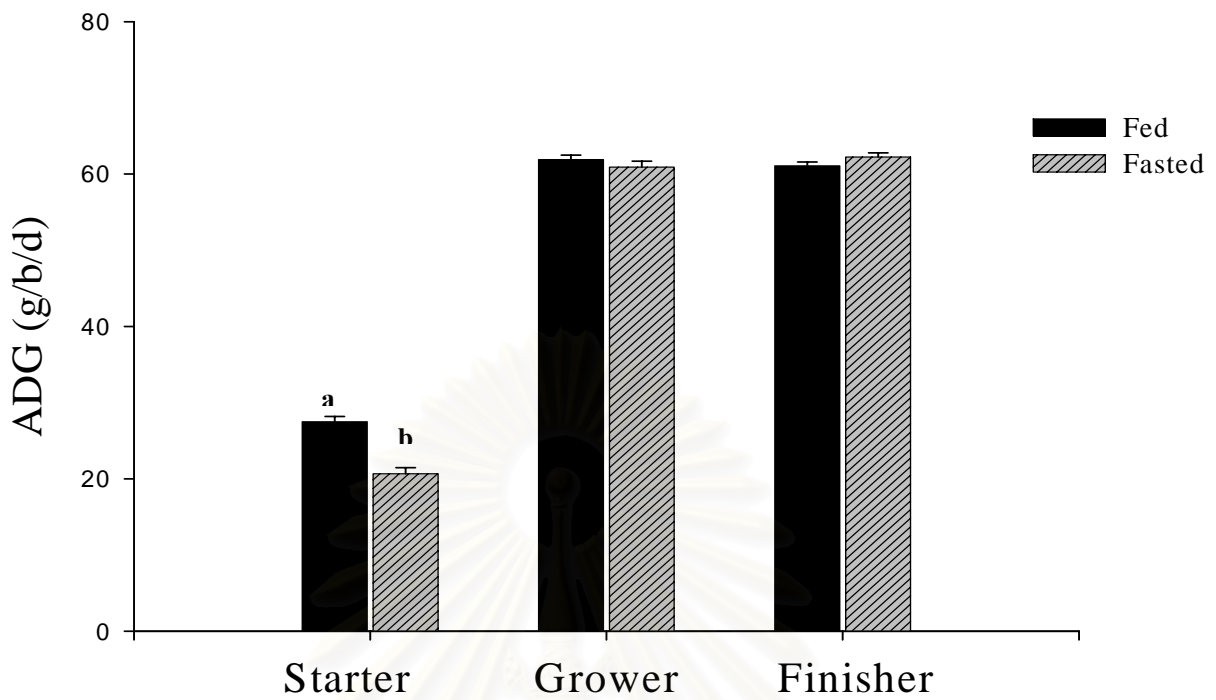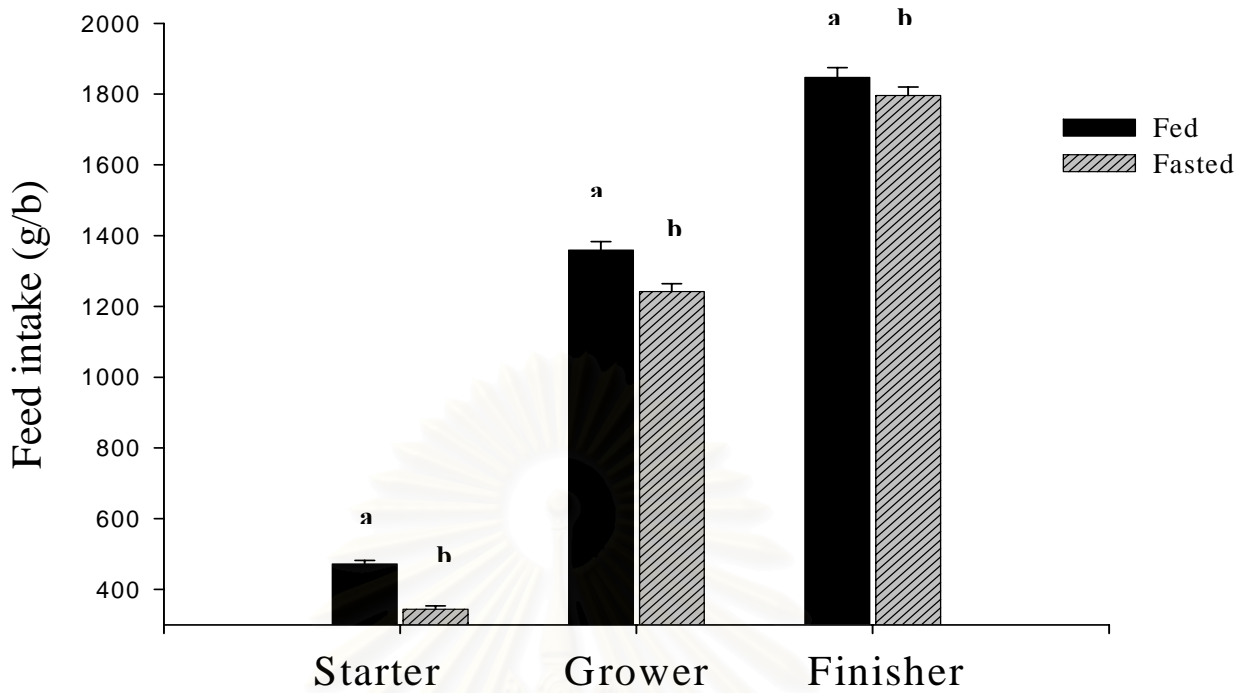a,b,c Different superscript mean significantly difference in the same period (p<0.05)

For the overall period of trial (days 1-42 of age), growth performance are shown in Table 7. Fed chicks had significantly ($p < 0.05$) higher final body weight (approximately 90.7 g/b) than fasted chicks. In addition, the body weight gain and ADG of fed chicks were 98.6 g/b and 2.4 g/b/d greater than fasted chicks, respectively. There were no significant difference from selenium supplementation on the final body weight but it was found selenium supplemented groups had slightly ($p < 0.05$) higher final body weight than control (approximately 73.9 g/b) and IOS group (approximately 78.2 g/b). In contrast, fed chicks that received OS (treatment 2) had significantly ($p < 0.05$) higher body weight gain than fasted chicks (treatments 4, 5 and 6) (Figure 28). Fasted chicks for 48 h had significantly ($p < 0.05$) lower feed intake (approximately 290 g/b) than chicks fed immediately after allocation (Figure 29). Fasted chicks that received OS (treatment 5) had slightly higher feed intake and DFI than IOS supplemented group (treatment 6) and control group (treatment 4). In addition, fasted chicks had significantly ($p < 0.05$) better FCR than fed chicks (Figure 30) and OS supplementation had slightly better FCR than control and IOS supplementation. In contrast, fasted chicks that received OS (treatment 5) had significantly better FCR than fed chicks (treatments 1, 2 and 3) and slightly better FCR than other fasted chicks (treatments 4 and 6). It was found that fed chicks had higher mortality rate than fasted chicks. Furthermore, chicks that received diet supplemented with OS (main effect) and control had significantly ($p < 0.05$) higher body weight uniformity than IOS supplemented chicks and fasted chicks had significantly ($p < 0.05$) lower body weight uniformity than fed chicks (Table 7).

Table 7 Effect of posthatch feeding or fasting on growth performance of broilers fed practical diet with supplemented organic or inorganic selenium (Days 1-42)

Group	Feeding regimen	Se supplementation (ppm)	Initial body wt. (g/b)	Final body wt. (g/b)	Body wt. gain (g/b)	ADG (g/b/d)	Feed intake (g/b)	DFI (g/b/d)	Feed conversion ratio	Mortality (%)	Culled* (%)	Body wt. uniformity (%)
1	Fed	nil	43.1	2105.3[ab]	2062.2[ab]	49.1[ab]	3600.9[a]	85.7[a]	1.748[a]	5.63	11.25	90.73
2	Fed	0.2 as OS (Sel-Plex)	43.0	2225.5[a]	2182.1[a]	52.0[a]	3729.8[a]	88.8[a]	1.710[bc]	3.75	10.63	91.84
3	Fed	0.2 as IOS (Na_2SeO_3)	43.0	2131.7[ab]	2088.7[ab]	49.7[ab]	3632.6[a]	86.5[a]	1.741[ab]	3.75	10.94	89.78
4	Fasted	nil	42.9	2060.6[b]	2017.7[b]	48.0[b]	3371.3[b]	84.3[b]	1.672[d]	1.88	10.94	89.88
5	Fasted	0.2 as OS (Sel-Plex)	43.1	2084.2[b]	2041.7[b]	48.6[b]	3396.0[b]	84.9[b]	1.664[d]	2.81	11.25	89.53
6	Fasted	0.2 as IOS (Na_2SeO_3)	42.9	2025.5[b]	1982.6[b]	47.2[b]	3331.4[b]	83.3[b]	1.681[cd]	2.81	10.94	88.85
Pooled SEM			0.092	40.47	40.46	0.96	54.60	1.45	0.01	0.21	0.05	0.74
CV, %			0.60	5.44	5.55	5.55	4.41	0.04	1.85	30.72	4.38	0.02

Source	df											
		-- p-value--										
Treatment diet	5	0.349	0.039	0.039	0.040	0.001	0.001	0.001	0.285	0.778	0.097	
Feeding regimen (A)	1	0.284	0.008	0.008	0.008	0.001	0.040	0.001	0.024	0.747	0.045	
Se supplementation (B)	2	0.505	0.129	0.130	0.131	0.250	0.554	0.063	0.969	0.901	0.038	
(A) x (B)	2	0.562	0.453	0.452	0.543	0.579	0.883	0.365	0.318	0.502	0.875	

Main effect

Feeding regimen

			Initial body wt.	Final body wt.	Body wt. gain	ADG	Feed intake	DFI	Feed conversion ratio	Mortality	Culled*	Body wt. uniformity
Fed			43.03	2155.5[a]	2112.4[a]	50.3[a]	3656.2[a]	87.0[a]	1.733[a]	4.38[a]	10.94	90.78[a]
Fasted 48 h			42.95	2056.8[b]	2013.8[b]	47.9[b]	3366.2[b]	84.0[b]	1.672[b]	2.50[b]	11.04	89.42[b]

Se supplementation

			Initial body wt.	Final body wt.	Body wt. gain	ADG	Feed intake	DFI	Feed conversion ratio	Mortality	Culled*	Body wt. uniformity
nil			42.98	2082.9	2040.0	48.6	3486.1	84.8	1.710	3.75	11.09	90.30[ab]
0.2 ppm as OS (Sel-Plex)			43.05	2156.8	2113.7	50.3	3565.5	86.9	1.686	3.28	10.94	90.68[a]
0.2 ppm as IOS (Na_2SeO_3)			42.94	2078.6	2035.7	48.5	3482.0	84.9	1.711	3.28	10.94	89.32[b]

[a,b] Means within column for treatment diet or for each main effect with no common superscript differ significantly ($p < 0.05$)

 * For tissue sample collection and culling.
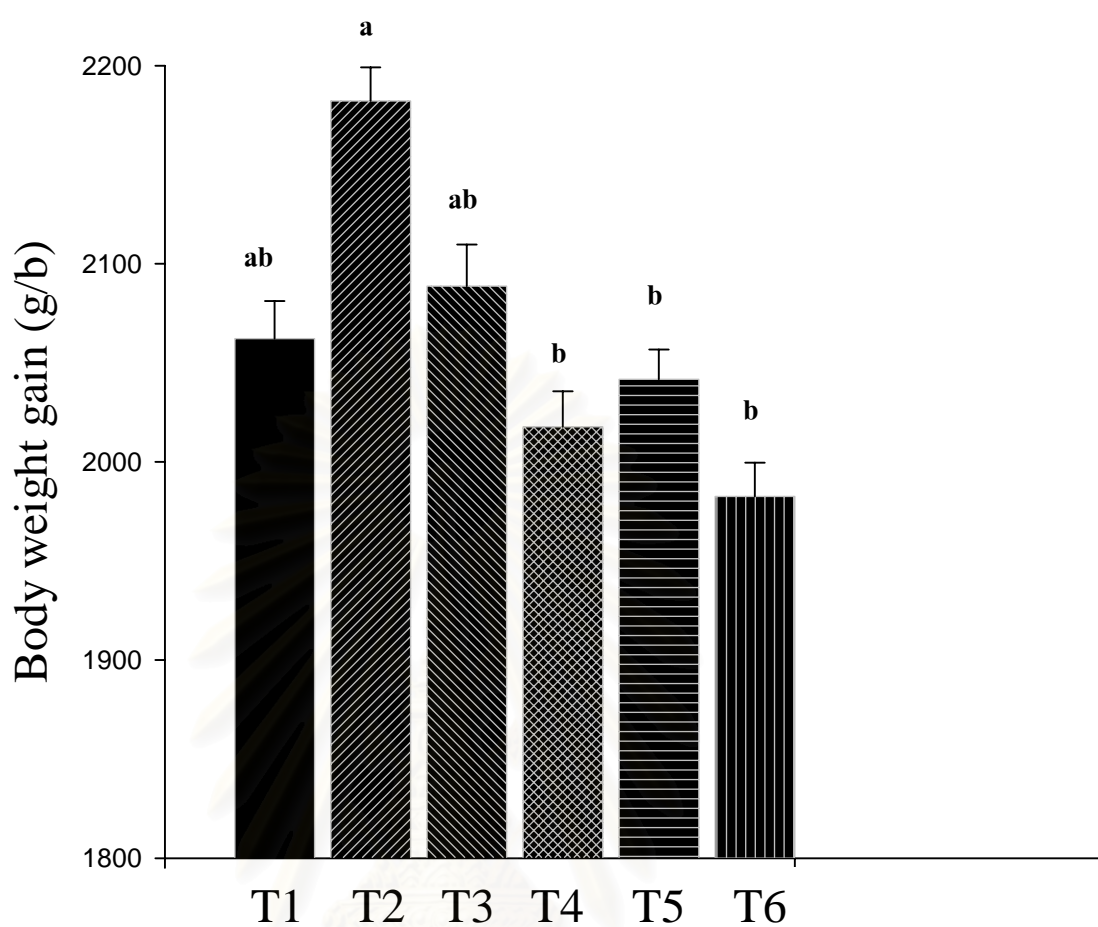
^{a,b} Different superscripts mean significantly difference (p<0.05)

Figure 28 Effect of posthatch feeding or fasting on body weight gain (g/b) of broiler fed on practical diet supplemented with organic or inorganic selenium (days 1-42).
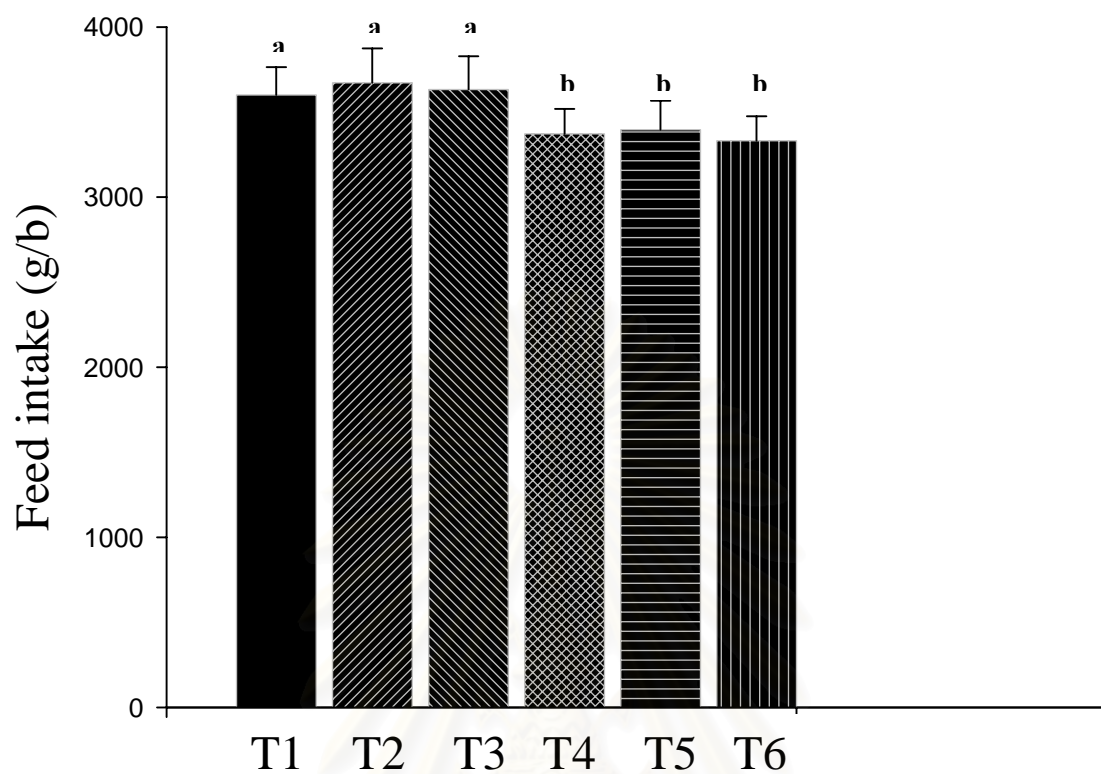
a,b Different superscripts mean significantly different (p<0.05)

**Figure 29** Effect of posthatch feeding or fasting on feed intake (g/b) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 1-42).
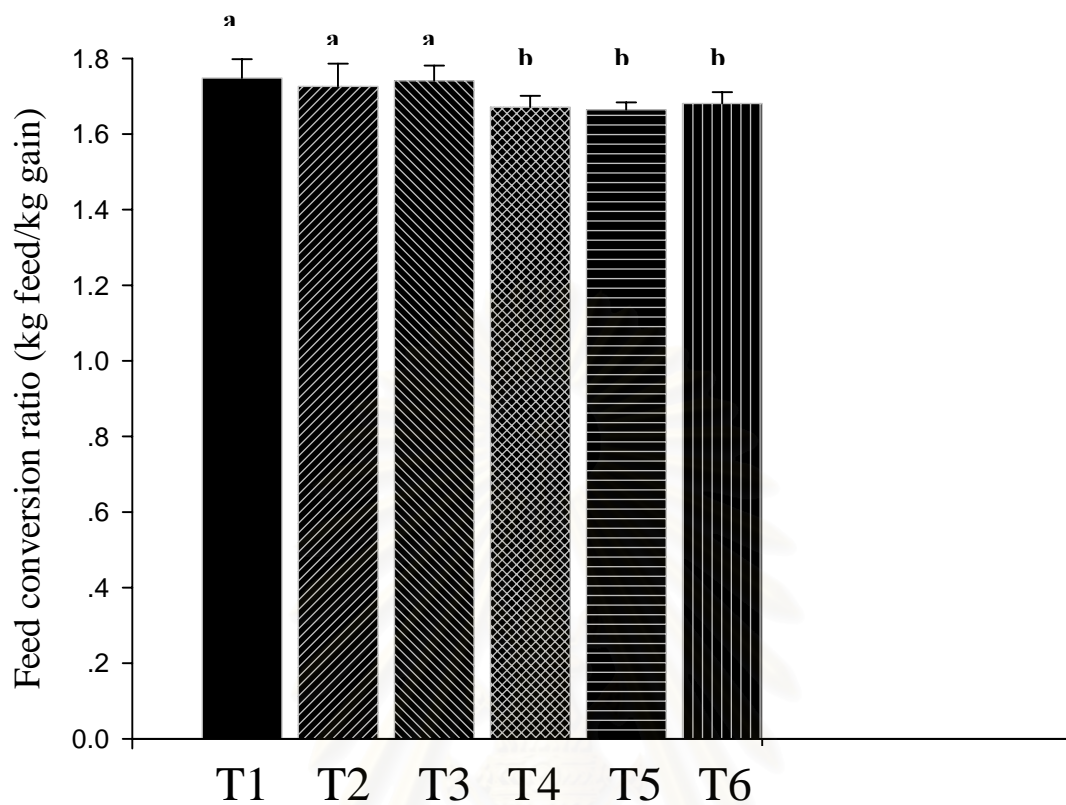
a,b Different superscripts mean significantly different (p<0.05)

Figure 30 Effect of posthatch feeding or fasting on feed conversion ratio (kg feed/kg gain) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 1-42).

Effect on liver glutathione peroxidase activity

The glutathione peroxidase (GSH-Px) activities in liver are shown in Table 8. At days 14 and 21 of age, there were no significant difference ($p > 0.05$) on GSH-Px activity in each treatment diet but chicks supplemented with selenium (treatments 2, 3, 5 and 6) had slightly higher GSH-Px activity than control groups (treatments 1 and 4). In addition, there were no significantly difference on GSH-Px activity from feeding regimen, but it was found that at days 7, 21 and 42 of age, fasted chicks had lower GSH-Px activity. On the contrary, at days 14, fasted chicks had slightly higher GSH-Px activity than fed chicks. Selenium supplementation affected GSH-Px activity ($p < 0.05$). Chicks that received diet supplemented with OS (days 7 of age) had significantly ($p < 0.05$) higher GSH-Px activity than control. At days 7 and 42 of age, selenium supplemented groups had significantly ($p < 0.05$) higher GSH-Px activities than control groups. Difference was found on GSH-Px activity due to effect from treatment diet (Figure 30). Selenium supplemented groups (treatment 2, 3, 5, 6) had significantly ($p < 0.05$) higher GSH-Px activity than control groups (treatments 1, 4) and fed control (treatment 1) had significantly ($p < 0.05$) higher GSH-Px activity than fasted control (treatment 4).

Effect on liver catalase activity

The catalase (CAT) activities are shown in Table 9. At days 7, 14, 21 and days 42 of age, there were no significant difference ($p > 0.05$) on CAT activity in both treatment diets and main effect (feeding regimen and selenium supplementation). In contrast, fasted chicks that received selenium (treatments 5 and 6) had slightly higher CAT activity than fed and fasted control (treatments 1 and 4) in every period. Furthermore, at days 14, 21 and 42 of age, fed chicks had slightly greater ($p > 0.05$) CAT activity than fasted chicks except at day 7 of age. Moreover, at days 7, 14, 21 and 42 of age, both forms of selenium supplemented chicks (treatments 2, 3, 5, and 6) had slightly higher ($p > 0.05$) CAT activity than chicks received nil (treatments 1 and 4).

Table 8 Effect of feeding regimen and selenium supplementation on liver glutathione peroxidase activity of broiler fed practical diet (Day 1-42).

	Treatment		Glutathione peroxidase activity				
Group	Feeding regimen	Se supplementation (ppm)			(munit / mg protein)		
			Day1	Day7	Day14	Day21	Day42
1	Fed	nil	202.4	381.1	397.2	435.7	458.7[b]
2	Fed	0.2 as OS (Sel-Plex)	202.4	430.0	440.4	492.7	494.2[a]
3	Fed	0.2 as IOS (Na_2SeO_3)	202.4	398.0	433.7	493.3	528.5[a]
4	Fasted	nil	202.4	349.0	420.2	437.0	450.6[b]
5	Fasted	0.2 as OS (Sel-Plex)	202.4	400.1	436.4	472.2	502.2[a]
6	Fasted	0.2 as IOS (Na_2SeO_3)	202.4	395.7	448.8	482.9	484.1[a]
Pooled SEM				22.56	34.35	27.14	14.91
CV, %				13.51	18.79	13.60	7.73
				----------p-value----------			
Source		df					
Treatment diet		5		0.130	0.952	0.263	0.001
Feeding regimen (A)		1		0.154	0.676	0.620	0.369
Se supplementation (B)		2		0.025	0.475	0.082	0.006
A X B		2		0.554	0.897	0.901	0.471
Main effect							
Feeding regimen							
Fed				403.0	423.8	473.9	502.8
Fasted 48 h				381.6	435.2	464.0	479.0
Se supplementation							
nil				365.0[b]	408.7	436.4	454.7[b]
0.2 ppm as OS (Sel-Plex)				415.1[a]	438.4	482.5	498.2[a]
0.2 ppm as IOS (Na_2SeO_3)				396.8[ab]	441.3	488.1	506.3[a]

[a,b,c] Mean within column for treatment diet or for each main effect with no common superscript differ significantly (p<0.05)

Table 9 Effect of feeding regimen and selenium supplementation on liver catalase activity of broiler fed practical diet

(Day 1-42).

Treatment			Catalase activity (unit / mg protein)				
Group	Feeding regimen	Se supplementation (ppm)	Day1	Day7	Day14	Day21	Day42
1	Fed	nil	46.11	58.03	57.21	66.10	84.18
2	Fed	0.2 as OS (Sel-Plex)	46.11	74.24	62.71	78.62	92.16
3	Fed	0.2 as IOS (Na$_2$SeO$_3$)	46.11	70.90	66.77	72.18	89.80
4	Fasted	nil	46.11	70.99	59.20	66.98	81.99
5	Fasted	0.2 as OS (Sel-Plex)	46.11	81.50	59.41	68.52	89.76
6	Fasted	0.2 as IOS (Na$_2$SeO$_3$)	46.11	76.01	58.47	68.98	90.97
Pooled SEM				6.59	4.51	6.31	5.60
CV, %				25.92	21.05	25.43	17.97
				------------------------------------*p-value*------------------------------------			
Source		df					
Treatment diet		5		0.312	0.903	0.924	0.888
Feeding regimen (A)		1		0.139	0.483	0.567	0.747
Se supplementation (B)		2		0.156	0.700	0.708	0.161
A X B		2		0.830	0.630	0.804	0.889
Main effect							
Feeding regimen							
Fed				67.73	62.23	72.30	88.71
Fasted 48 h				76.17	59.03	68.16	87.58
Se supplementation							
nil				64.51	58.2	66.54	83.08
0.2 ppm as OS (Sel-Plex)				77.86	61.06	73.57	90.97
0.2 ppm as IOS (Na$_2$SeO$_3$)				73.46	62.62	70.58	90.39
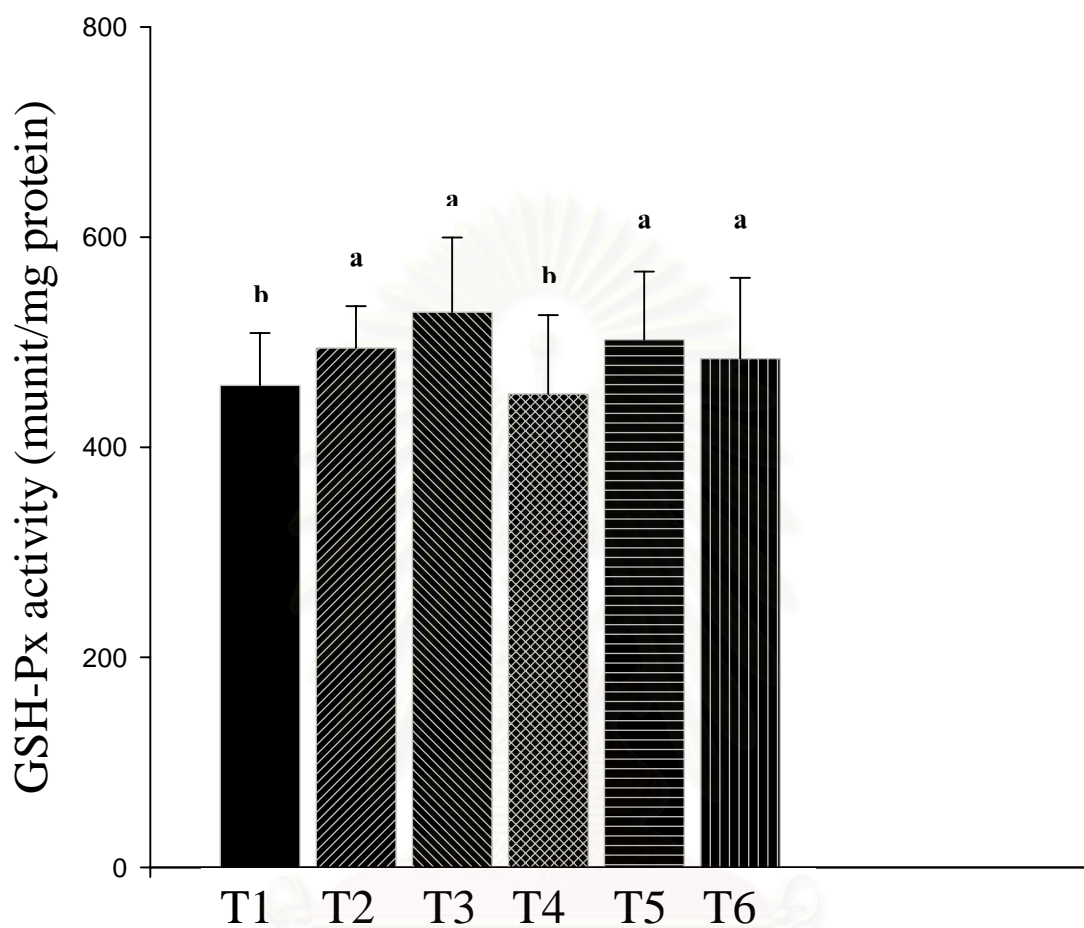
a,b Different superscripts mean significantly different (p<0.05)

Figure 31 Effect of posthatch feeding or fasting on glutathione peroxidase activity (munit/mg protein) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 42)

Effect on liver superoxide dismutase activity

The superoxide dismutase (SOD) activities are shown in Table 10. It was found that at days 14 and 42 of age, selenium supplemented groups (treatments 2, 3, 5, 6) had significantly ($p < 0.05$) higher SOD activity than control groups (treatments 1, 4) (Figure 31). In contrast, at days 7 and 21 of age, there were no significant difference on SOD activity in each treatment group, but it was found that selenium supplemented groups had slightly higher ($p > 0.05$) SOD activity than control. In addition, fasted chicks that received OS and IOS (treatments 5 and 6) had slightly ($p > 0.05$) higher SOD activity than fed and fasted control groups (treatments 1 and 4). Moreover, there were no significantly ($p > 0.05$) different on SOD activity from the effect of feeding regimen. Fed chicks had slightly higher SOD activity than fasted chicks (days 7 and 14 of age). On the contrary, at days 21 and 42 of age fasted chicks for 48 h had slightly ($p > 0.05$) higher SOD activity than fed chicks.

Table 10 Effect of feeding regimen and selenium supplementation on liver superoxide dismutase activity of broiler fed practical diet.

(Day 1-42)

Group	Feeding regimen	Se supplementation (ppm)	Day1	Day7	Day14	Day21	Day42
	Treatment				Superoxide dismutase activity (unit / mg protein)		
1	Fed	nil	7.14	9.91	10.46[b]	13.84	17.37
2	Fed	0.2 as OS (Sel-Plex)	7.14	12.14	13.24[a]	15.21	19.24
3	Fed	0.2 as IOS (Na$_2$SeO$_3$)	7.14	12.10	13.54[a]	14.95	19.64
4	Fasted	nil	7.14	9.95	9.82[b]	16.82	18.21
5	Fasted	0.2 as OS (Sel-Plex)	7.14	11.92	12.67[a]	18.24	21.11
6	Fasted	0.2 as IOS (Na$_2$SeO$_3$)	7.14	11.44	12.81[a]	17.49	20.79
Pooled SEM				0.86	0.29	0.58	0.42
CV, %				25.77	8.49	14.46	9.68

--- *p-value* ---

Source	df						
Treatment diet	5			0.762	0.001	0.121	0.880
Feeding regimen (A)	1			0.789	0.152	0.059	0.105
Se supplementation (B)	2			0.225	0.001	0.662	0.042
A X B	2			0.949	0.986	0.983	0.821

Main effect

Feeding regimen

				Day7	Day14	Day21	Day42
Fed				11.38	12.41	14.66	18.75
Fasted 48 h				11.12	11.77	17.51	20.04

Se supplementation

				Day7	Day14	Day21	Day42
nil				9.95	10.14[b]	15.33	17.79[b]
0.2 ppm as OS (Sel-Plex)				12.03	12.96[a]	16.72	20.17[a]
0.2 ppm as IOS (Na$_2$SeO$_3$)				11.77	13.18[a]	16.22	20.22[a]

[a,b] Mean within column for treatment diet or for each main effect with no common superscript differ significantly ($p < 0.05$)

a,b Different superscripts mean significantly different (p<0.05)

Figure 32 Effect of posthatch feeding or fasting on superoxide dismutase activity (unit/mg protein) of broilers fed on practical diet with supplemented organic or inorganic selenium (days 14).
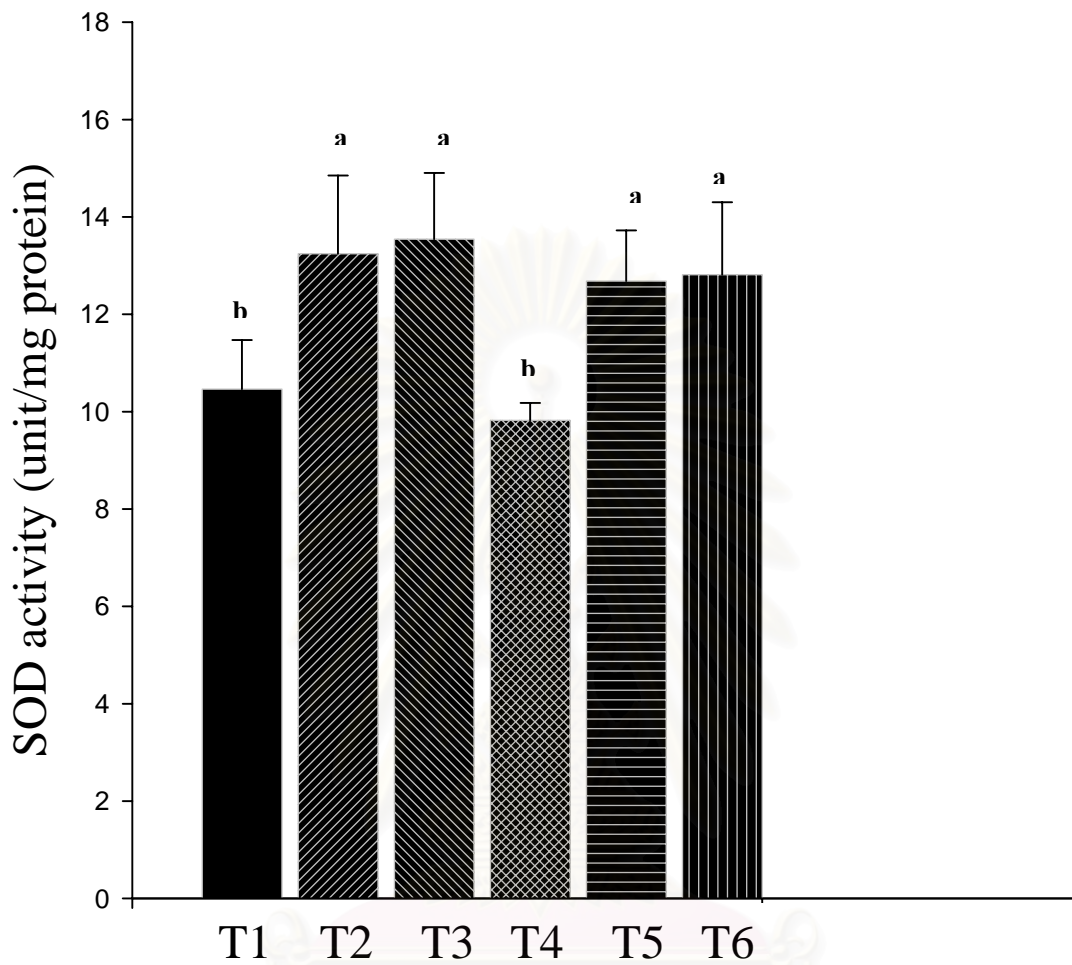
Effect on small intestinal disaccharidase activities

Maltase

The alteration in maltase activity of jejunal mucosa at day 21 of age is shown in Table 11. It was found that there was no significant difference on maltase activities from the effect of feeding regimen and selenium supplementation. In contrast, fed chicks immediately allocation finished had slightly ($p > 0.05$) higher maltase activity than fasted chicks for 48 h after allocation finished. In addition, it was found that OS supplementation had slightly higher ($p > 0.05$) maltase activity than control approximately 51.42 unit/mg protein and IOS supplementation had slightly higher ($p > 0.05$) maltase activity than control. Moreover it was found that fasted chicks that received OS (treatment 5) and IOS (treatment 6) had slightly higher maltase activity than fed (treatment 1) and fasted control (treatment 4).

Sucrase

The sucrase activities of jejunal mucosa at day 21 are shown in Table 11. It was found that no significant difference among treatment group and there were no significant difference on sucrase activity from the main effect (feeding regimen and selenium supplementation). In contrast, it was found that OS supplementation had slightly ($p > 0.05$) higher sucrase activity than control approximately 21.13 unit/mg protein and IOS supplementation had slightly higher ($p > 0.05$) sucrase activity than control. In addition, it was found that OS supplementation (treatment 5) and IOS supplementation in fasted chicks (treatment 6) had slightly ($p > 0.05$) higher maltase activity than fed (treatment 1) and fasted control chicks (treatment 4).

Effect on serum tri-iodothyronine (T3) level.

Serum T3 levels are shown in Table 11. At day 14, there was an interaction between two studied factors (feeding regimen and selenium supplementation) for T3 levels. Chicks that supplemented with selenium (treatments 2,3,5,6) had significantly ($p<0.05$) greater T3 levels than chicks that received nil (treatments 1, 4). At days 14 of age, it was found that feeding regimen (fed or fasted) affects the T3 levels, fasted chicks had significantly ($p<0.05$) higher serum T3 levels than fed chicks (days 14 of age). T3 levels reached the highest level at days 7 of age and then gradually fall through days 21 of age. There was no significant difference on T3 level at days 7 and 21 of age but OS supplementation birds had slightly ($p>0.05$) higher T3 levels than the control. On the contrary, IOS supplementation had slightly lower T3 levels than control (at days 7). In addition, at days 21 of age, it was found that fasted chicks that received OS had slightly higher T3 levels than fed chicks and other fasted chicks.

Table 11 Effect of feeding regimen and selenium supplementation on disaccharidase activities and thyroid hormone (T3) level of broiler fed practical diet.

	Treatment		Disaccharidase activities (days 21)		Thyroid hormone (T3) level			
Group	Feeding regimen	Se supplementation (ppm)	(unit / mg protein)		(ng / dl)			
			Maltase	Sucrase	Day1	Day7	Day14	Day21
1	Fed	nil	201.08	66.1	116.95	206.87	116.58	111.19
2	Fed	0.2 as OS (Sel-Plex)	284.11	91.98	116.95	217.87	128.66	110.54
3	Fed	0.2 as IOS (Na_2SeO_3)	231.89	87.86	116.95	221.12	121.41	106.13
4	Fasted	nil	213.18	71.89	116.95	194.37	124.97	103.53
5	Fasted	0.2 as OS (Sel-Plex)	233.00	88.26	116.95	201.25	141.00	126.75
6	Fasted	0.2 as IOS (Na_2SeO_3)	261.77	84.25	116.95	172.95	161.81	119.87
Pooled SEM			22.49	9.66		18.52	10.97	9.29
CV, %			26.79	33.46		26.31	23.45	23.26
					----------p-*value*----------			
Source		df						
Treatment diet		5	*0.148*	*0.358*		*0.559*	*0.096*	*0.507*
Feeding regimen (A)		1	*0.872*	*0.947*		*0.092*	*0.002*	*0.195*
Se supplementation (B)		2	*0.090*	*0.086*		*0.704*	*0.002*	*0.518*
A X B		2	*0.203*	*0.842*		*0.497*	*0.005*	*0.769*
Main effect								
Feeding regimen								
Fed			239.03	81.98		215.29	122.22[b]	103.84
Fasted 48 h			235.98	81.46		186.99	143.83[a]	116.72
Se supplementation								
nil			207.13	68.99		200.62	115.12[b]	101.94
0.2 ppm as OS (Sel-Plex)			258.55	90.12		209.56	134.83[a]	115.89
0.2 ppm as IOS (Na_2SeO_3)			246.83	86.05		193.23	149.12[a]	113.00

[a,b] Mean within column for treatment diet or for each main effect with no common superscript differ significantly (p<0.05)

CHAPTER V

DISCUSSION AND CONCLUSION

Maternal diet composition is a major determinant of antioxidant system development in the chicks during embryogenesis and early postnatal development. Selenium is another important determinant of antioxidant system efficiency. Selenium content in the egg depends on its concentration in hen's diet, and also on the form of dietary selenium used, since organic selenium deposit more efficiency in the egg than inorganic selenium (Paton *et al*, 2002). There was also an indication that selenium can be transferred to chicks embryo tissues from the egg content (Surai, 2000). Embryonic tissues comprise phospholipids that contain large amounts of highly polyunsaturated fatty acids, particularly arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acid (Noble *et al*, 1993), which was susceptible to lipid peroxidation. An optimal antioxidant status of newly hatched chicks were required for their protection against damaging effects of free radical and product of their metabolism (Surai, 2000). During transportation from hatcheries to farms or delay feed intake after hatch are stressor that caused free radical generation (Noy *et al*, 2001). A considerable degree of antioxidant defense was required to reduce peroxidation. Hydrogen peroxide formed by SOD was toxic and must be removed from the cell. Two enzymes, GSH-Px and CAT were responsible for reduction of H_2O_2 to water (Surai, 1999). In modern poultry production, it is common that chicks are usually access to feed within 48 h after hatching. Time to the first feed can be longer in some country such as PRC (People Republic China) that farms are remote from the hatchery. In addition, poor management at early stage of raising the chicks, can lead to delay first access to feed. A previously belief that remnant egg yolk can nourish the chick up to 48-72 h after hatching is not applicable to modern poultry production (Uni *et al*, 1998) since most broiler were fast growing and needed early energy supply. The growth advantage obtained by early feeding is maintained through market age (Noy and Sklan, 2001)

Starter period (days 1-14)

Three antioxidant enzymes were examined in chicks from parent stock were not supplemented with selenium in diet. The result showed that OS supplemented chicks had higher GSH-Px activity than control. Organic selenium as selenomethionine is the storage proteins in liver and muscle which is important for GSH-Px synthesis. Thus chicks that received diet supplemented with selenium had higher GSH-Px activity than chicks that received basal diet. In addition, selenium supplemented groups had significantly higher SOD activity than control, which related with GSH-Px for reactive oxygen scavenging. The selenium level of selenium supplemented groups suggested that selenium level were much higher than the recommendation of National Research Council (1994) (0.15 mg/ kg diet). However, there were no difference on CAT activity among treatment groups, which catalyzed the same substrate with GSH-Px. More than thirty selenoproteins have been identified, among these, iodothyronine deiodinase was shown to be a functional selenoprotein. This enzyme is required to catalyze thyroxine to its active form, tri-iodothyronine (T3). Thyroid functions were known to be altered by many environmental factors, such as energy intake, ambient temperature and dietary composition (Mcnabb, 2000). In previous studies, selenium deficiency caused decreased serum T3 and this might be a reason why growth was impaired in selenium deficiency birds (Jianhua *et al*, 2000). In this study, serum T3 levels reached the highest levels at days 7 of age then decreased through days 21 of age. High level of T3 at early ages may be used to stimulate growth and development. It was found that there were an interaction between main effects (selenium supplementation, feeding regimen) at days 14 of age. High level of serum T3 was induced by selenium supplementation (days 14). In addition, fasted chicks had higher serum T3 levels than fed chicks, which affected growth performance. Fasted chicks had markedly decrease feed intake and DFI. It had been previous established that there was a significantly linear correlation exists between serum T3 and feed intake (Yahav *et al*, 1996; Yahav *et al*, 1998). Feed utilized after hatching in fed chicks led them to express feeding capability according to their age and requirement for growth and maintenance (Noy *et al*, 2001), so fed chicks had higher body weight gain and ADG than fasted chicks. On the contrary, fasted chicks can use feed more efficient than fed chicks. It is shown that the FCR of fasted chicks was

significantly better than fed chicks at the same age. Fasted chicks for more 48 h posthatch had delayed small intestinal development, probably by reduction of the small intestinal weight, and length, which affected feed utilization (Geyra *et al*, 2001; Iji *et al*, 2001a). Since fasted chicks had lower feed intake, thus they had low capability to retain the amount of feed in the small intestine. Therefore the small amount of feed in the intestine were better digestion and absorption of nutrients.

Grower period (days 15-28)

Since the development of the gastrointestinal tract was mature in this period, thus the late development of intestine in chicks fasted at early age exacerbated intestinal weight and enzyme production (Iji *et al*, 2001b). The activity of disaccharidase enzymes, maltase and sucrase in various treatments were examined within grower period. It was found that chicks that given diet immediately after arriving had slightly higher maltase activity comparison to fasted chicks for 48 h. It is possible that delay feed intake at early posthatching suppressed small intestinal development. However, the adaptive response of the small intestine to fasting influenced subsequent digestive and absorptive function and small intestinal development (Noy and Sklan, 2001). Fasting at early posthatch period depresses small intestinal enterocyte proliferation and migration, crypt and villus development, in particular in the duodenum and jejunum led to decreased small intestinal enzyme production (Iji *et al*, 2001b). These results indicated that early access to feed is very important for posthatch chicks development (Geyra *et al*, 2001). Since the small intestine developed rapidly at early ages in chickens when fed immediately posthatch period, fasting chicks for 48 h at hatch retarded small intestinal growth and development. Uni *et al* (1998) who reported fasting chicks for 36 h decreased villus volume, crypt depth and caused jejunal villi damage. Selenium supplemented chicks (both fed and fasted) had slightly higher maltase and sucrase activities than control groups. It may be possible that selenium plays an important role in antioxidant defense which can protect the cell from injury caused by peroxides and helps to support the strong integrity of mucosal cells in the small intestine. Fasted chicks had lower in body weight gain, ADG, feed intake and DFI. On the contrary, chicks that received diet supplemented with OS (main effect) had

better ADG, FCR and uniformity than chicks that received IOS. OS had high potential for enhance the antioxidant enzyme status and prevent peroxidative damage which may be occur during fasting than IOS. This induced better feeding utilized and better growth in OS supplemented chicks.

Finisher period (days 29-42)

The final body weight, DFI and uniformity were still higher in fed chicks. These results supported that maximal body weight and feed intake might be attainted during early posthatch period when chicks were fed. Thus, fasted chicks for 48 h could not achieve feed intake and body weight gain compared to fed chicks in these periods (Bigot *et al*, 2003) but fasted chicks had better FCR than fed chicks. The higher antioxidant status in selenium supplemented chicks could help the better growth than chicks that received basal diet, which had lower selenium concentrations. However, compensatory growth were not found in fasted chicks in response to delayed feed intake. Compensatory growth, is referred to the rapid weight gain, that usually follows a period of reduced nutrient intake of chickens. When chicks were placed back on a high quality diet, they grew faster than control chicks. Since the chicken will be underweight for its age, increased nutrient intake is usually leading to a fairly rapid and efficient gain in body weight. The pattern of compensatory growth is influenced by the optimal age and maturity of the animal (Deaton *et al*, 1973).

Overall period (days 1-42)

Fed chicks had significantly higher final body weight, body weight gain and ADG, DFI, mortality rate and flock uniformity than fasted chicks. On the contrary fasted chicks had better FCR compared to fed chicks. The high mortality rate in fed chicks was induced by high feed intake that resulted in an increased heat production in the body, which induced metabolic stress especially in week 5-6 of age. However, chicks that received diet supplemented with selenium trend to had lower mortality rate than control. These results show that selenium are required to prevention of free radical generation from stressor via the high activity of GSH-Px. This result was similar to

Jianhua *et al* (2000), who reported that selenium supplementation increased GSH-Px activity.

In conclusion, fasting chicks for 48 h and were decreased body weight, feed intake and flock uniformity, but that chicks had better FCR and mortality rate compared to fed chicks. Selenium supplementation in both fed and fasted chicks that came from parent stock without selenium in diet helped to increase GSH-Px (days 7 and 42 of age) and SOD 14 and 42 of age, enhanced T3 levels in the second week, tended to increase disaccharidase activities at days 21 of age and improved growth performance. Organic selenium supplemented chicks had better growth performance (ADG, FCR at days 15-28 of age and flock uniformity; at days 1-42 of age) and uniformity than inorganic selenium supplemented chicks.
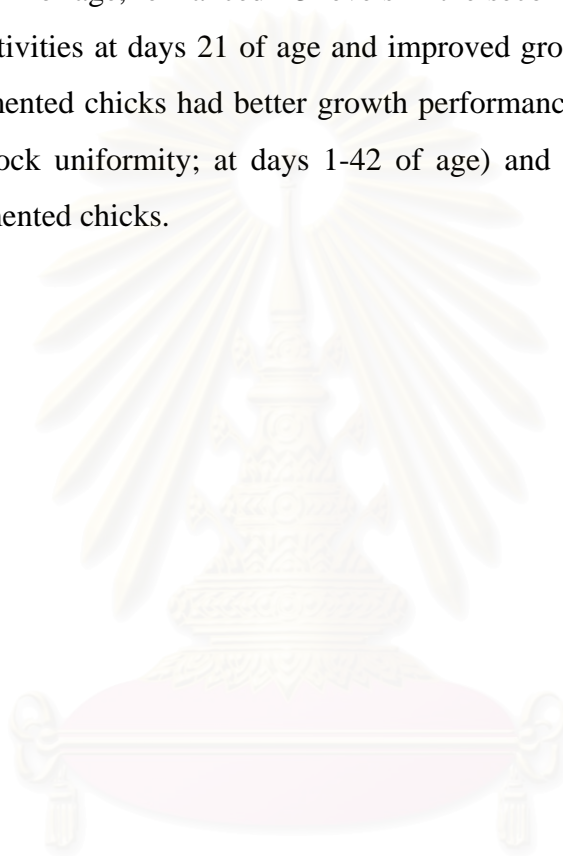
REFERENCES

Aebi, H. 1984. Catalase in vitro. Methods in enzymology. 105:121-126.

Armstrong, D. 1998. Free radical and antioxidant protocols. Humana Press. New Jersey :299-310.

Arthur, JR., Nicol, F. and Beckett, JG. 1990. Hepatic iodothyronine 5' deiodinase.Biochem.J. 272:537-540.

Beckett, GJ., Beddows, SE., Morrice, PC., Nicol, F. and Arthur, JR.1987. Inhibition of hepatic deiodination of thyroxine is caused by selenium deficiency in rats. Biochem.J. 248:443-447.

Beckett, GJ., Russell, A., Nicol, F., Sahu, P., Wolf, CR. and Arthur, JR. 1992. Effects of selenium deficiency on hepatic type I 5-iodothyronine deiodinase activity and hepatic thyroid hormone levels in the rat. Biochem. J.282:483-486.

Beers, R.F. and Sizer, I.W. 1951. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Biol.Chem.194:133-140.

Bigot, K., Mignon, S.G., Picard, M. and Tesseraud, S. 2003. Effect of delay feed intake on body, intestine and muscle development in neonate broilers. Poult. Sci. 82:781-788.

Chu, F.F., Doroshow, J.H. and Esworthy, R.S. 1993. Expression of characterisation and tissue distribution of a new cellular selenium dependent glutathione peroxidase, GSH-Px-G1. J. Biol. Chem. 268:2571-2576.

Colnago, G.L.,Gore, L., Jensen, L.S., and Long, P.L. 1982. Amelioration of pale bird syndrome in chick by vitamin E and selenium. Avian Dis. 27(1):312-323.

Dalhqvist, A.1968. Assay of intestinal disaccharidases. Anal.Biochem.22:99-107.

Deaton, J. W., Reece, F. N., Kubena, L. F., Colt, B. D. and J May, D. 1973, The ability of the broiler to compensate for early growth depression. Poult. Sci. 52:262-267.

Geyra, A. and Uni, Z. 2001. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br. J. Nutr. 86:53-61.

Geyra, A., Sklan, D. and Uni, Z. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. 80:776-782.

Hayashi, K., Tomita, Y., Shinagava, Y., and Hashizume, T. 1985. The rate of degradation of proteins of skeletal muscle in broiler and layer chickcen estimated by N-methylhistidine in excreta. Br. J. Nutr. 54:157-163.

Iji, P.A., Saki, A., and Tivey, D. R. 2001a. Body and intestinal growth of broiler chicks a commercial starter diet.1. Intestinal weight and mucosal development. Br.Poult.Sci. 42:505-513.

Iji, P.A., Saki, A., and Tivey, D. R. 2001b. Body and intestinal growth of broiler chicks a commercial starter diet. 2.Development and characteristics of intestinal enzyme. Br.Poult.Sci. 42:514-522.

Jensen, L. S., Conago, GL., Takahashi, K. and Akiba, Y. 1986. Selenium status and plasma thyroid hormone in chick. Biol. Trace. Elem. Res. 10:11-18 (Abstract)

Jianhua, H., Ohtsuka, A., and Hayashi, K. 2000. Selenium influences growth via thyroid hormone status in broiler chickens. Br J.Nutr. 84:727-732.

Lei, X. G., Dann, H. M., Ross, D. A., Cheng, W. S., Combs, G. F. and Roneker, K. R. 1998. Dietary selenium supplementation is required to support full expression of three selenium-dependent glutathione peroxidases in various tissues of weaning pigs. J Nutr. 128, 130-135.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 183:268-275.

Mccord, J. M. and Fridovich, I. 1969. Superoxide dismutase. J.Biol.Chem. 244 (22):6049-6055.

Mcnabb, F. M. 2000. Sturkie's avian physiology. Academic Press. New York. P:461-469.

Moran, E. T. and Reinhart, B. S. 1980. Poultry yolk sac amount and composition upon placement: effect of breeder age, egg weight, sex and subsequence change with feeding or fasting. Poult. Sci. 59: 1521-1528.

National Research Council. 1994. Nutritional requirement of poultry, 9 th ed. Washington, DC: National Academy press.

Noble, R.C., Cocchi, M. and Bath, H.M. 1993. α-Tocopherol absorption polyunsaturated fatty acid metabolism in the developing chick embryo. Br.Poult.Sci. 34:815-818.

Noguchi, T., Langevin, M. L., Combs, G.F. and Scott, M.L. 1973. Biological and histochemical studies of the selenium deficient pancreas in chicks. J. Nutr.103:444-453.

Noy, Y. and Sklan, D. 2001.Yolk and exogenous feed utilization in the posthatch chicks. Poult. Sci. 80:1490-1495.

Noy, Y. Geyra, A., and Sklan, D. 2001. The effect of early feeding on growth and small intestinal development in posthatch poult. Poult. Sci. 80:912-919.

Noy, Y., Uni, Z. and Sklan, D. 1996. Utilization of yolk on the newly hatch chicks. Br.Poult. Sci. 37:987-995.

Paton, N. D., Cantor, A. H., Pescatore, A.J., Ford, M. J. and Smith, C. A. 2002. The effect of dietary selenium source and level on the uptake of selenium by developing chick embryos. Poult. Sci. 81:1548-1554.

Rayman, M. P. 2002. Selenium: essential constituent of the human diet. Feeding times. 7(2):3-11.

Scott, N.Y., Nesheim, M. C. and Yong, R.J. 1982. Nutrition of the chicken. M.L.Scott and Associates, Publishers. New York: 347-355.

Sklan, D. and Noy, Y. 2000. Hydrolysis and absorption in the small intestine of posthach chick. Poult. Sci.79:1306-1310.

Smart, I. J., Embury, D. H., Barr, D. A., Sinclair, A. J. Karunajeewa, H., Ewind, I., Reece, R. L., Forsyth, W. M. and Hooper, P. T. 1985. Absence of a role for selenium deficiency in the runting stunting syndrome of broiler chickens in Australia. Avian dis. 29(4):1201-1211.

Surai, P.F. 1999. Tissue specific changes in the activities of antioxidant enzyme during the development of the chick embryo. Br.Poult.Sci. 40:397-405.

Surai, P.F. 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. Br.Poult.Sci. 41:235-243.

Surai, P.F. 2002. Selenium in poultry nutrition. 1. Antioxidant properties, deficiency and toxicity. World's Poult. Sci. 58: 333-347.

Surai, P. F., Sparks, N.H.C. and Noble, R. C. 1999a. Antioxidant system of the avian embryo:Tissue specific accumulation and distribution of vitamin E in the turkey embryo during development. Br. Poult. Sci.40:458-466.

Surai, P. F., Noble, R. C. and Speake, K.B. 1999b. Relationship between vitaminE content and susceptibility to lipid peroxidation in tissue of the newly hatch chick. Br. Poult. Sci. 40:406-410.

Tappel, A.L. 1978. Glutathione peroxidase and hydroperoxides. Methods in enzymology. 52:506-513.

Underwood, E.J. and Suttle, N.F. 1999. The mineral nutrition of livestock. CABI. New York: 421-475.

Uni, Z., Ganot, S. and Sklan, D. 1998. Posthatch development of mucosal function in the broiler small intestine. Poult.Sci.77:75-82.

Yahav, S., Plavnik, I., Rusel, M. and Hurwitz, S. 1998. Response of turkeys to relative humidity at high ambient temperature. Br. Poult. Sci. 39:340-345.

Yahav, S., Straschnow, A., Plavnik, I. and Hurwitz, S. 1996. Effect of diurnal cycle versus constant temperatures on chicken growth and food intake. Br. Poult. Sci. 37:43-54.

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