EFFECT OF GAMMA AMINOBUTYRIC ACID (GABA) FROM GERMINATED PADDY RICE IN RATION ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS IN BROILERS

KA TOLA

A thesis submitted in partial fulfillment of the requirements for

the Degree of Master of Science in Animal Science

at Mahasarakham University

August 2014

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This thesis was granted by
"Her Royal Highness Princess MAHA CHAKRI SIRINDHORN" Scholarship
Academic Year 2012

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincerest and respectful gratitude to Her Royal Highness Princess MAHA CHAKRI SIRINDHORN of Thailand who granted me the scholarship for my Master's Degree in Mahasarakham University. Her grant has been meaningful and crucial to me.

I would like to express my deep appreciation and sincere gratitude to my major advisor Associate Professor Dr. Songsak Champawadee for his supervision, encouragement and friendship throughout the course of my studies. My appreciation and gratitude are also extended to Dr. Doungnapa Promket, for her guidance and advice throughout the course of my thesis. Their support is very much appreciated. In addition, I would like to thank Associate Professor. Dr. Anuchita Moongngarm, the dean of the Faculty of Technology for her recommendations and support. I am very grateful to division of Animal Science, Faculty of Technology, Mahasarakham University for facilitating in the experimental. Moreover, I am grateful to my friends that helped me during my thesis.

Importantly, I would like to express my heartfelt gratitude to my beloved parents for their endless support in everything.

Ka Tola

ชื่อเรื่อง	ผลของระดับแกรมม่าอะมิโนบิวทีริค แอซิด (GABA) จากข้าวเปลือกงอก ในสูตรอาหารต่อสมรรถนะการเจริญเติบโต และคุณภาพซากของไก่กระทง
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บทคัดย่อ

การทดลองนี้มีวัตถุประสงค์ เพื่อศึกษาผลของระดับ แกรมม่าอะมิโนบิวทีริค แอซิด จากข้าวเปลือกงอกต่อสมรรถนะการเจริญเติบโต และคุณภาพซากในไก่กระทง ใช้แผนการทดลองแบบสุ่มสมบูรณ์ โดยใช้ไก่กระทงทั้งสองเพศอายุ 1 วันจำนวน 200 ตัว แบ่งเป็น 5 กลุ่มทดลองจำนวน 4 ซ้ำ แบ่งเป็น 20 กรงในแต่ละกรงมีไก่ 10 ตัวใช้ระยะเวลาทดลอง 42 วัน โดยกลุ่มการทดลอง คือเสริมระดับของ แกรมม่าอะมิโนบิวทีริค แอซิด จากข้าวเปลือกงอกในระดับ 0, 0.5, 1, 1.5 และ 2 มีลลิกรัมต่อกิโลกรัม ของอาหารตามลำดับ ผลการทดลองพบว่า ปริมาณแกรมม่าอะมิโนบิวทีริค แอซิดในข้าวเปลือกงอกมี 229.30 มีลลิกรัมต่อกิโลกรัม ปริมาณการกินได้ในสัปดาห์ที่ 1 ถึง 4 กับสัปดาห์ที่ 6 ไม่แตกต่างทางสถิติ ($P>0.05$) แต่ในสัปดาห์ที่ 5 พบว่าปริมาณ การกินได้แตกต่างทางสถิติ ($P<0.05$) โดยกลุ่มที่ได้รับ แกรมม่าอะมิโนบิวทีริค แอซิดในระดับ 2 มีลลิกรัมต่อกิโลกรัม มีปริมาณการกินได้สูงที่สุด นอกจากนี้อัตราการเจริญเติบโตพบว่า ในสัปดาห์ที่ 1, 3, 4 และ 5 ไม่แตกต่างทางสถิติ ($P>0.05$) แต่แตกต่างที่สัปดาห์ที่ 2 และที่ 6 ($P<0.05$) โดยในสัปดาห์ที่ 2 พบว่ากลุ่มได้รับ แกรมม่าอะมิโนบิวทีริค แอซิดในระดับ 2 มีลลิกรัมต่อกิโลกรัม มีอัตราการเจริญเติบโตสูงที่สุด และในสัปดาห์ที่ 6 กลุ่มที่ได้รับแกรมม่าอะมิโนบิวทีริค แอซิด ในระดับ 1 มีลลิกรัมต่อกิโลกรัม มีอัตราการเจริญเติบโตสูงที่สุด อัตราการเปลี่ยนอาหารเป็นน้ำหนักไม่แตกต่างทางสถิติ ($P>0.05$) นอกจากนี้เปอร์เซ็นต์ของน้ำหนักซากหลังเอาเครื่องในออก น้ำหนักเนื้อส่วนอก ส่วนน่อง ไขมันช่องท้อง ส่วนปีก ตับ ม้าม และหัวใจ ไม่มีความ แตกต่างกันทางสถิติ ($P>0.05$) แต่ เปอร์เซ็นต์ของ น้ำหนักซากหลังถอนขนสูงที่สุดในกลุ่มที่ได้รับแกรมม่าอะมิโนบิวทีริค แอซิด ในระดับ 2 มีลลิกรัมต่อกิโลกรัม ($P<0.05$) นอกจากนั้นยังพบว่าเปอร์เซ็นต์ส่วนสะโพกสูงที่สุดในกลุ่มที่ได้รับ แกรมม่าอะมิโนบิวทีริค แอซิดในระดับ 1 มีลลิกรัมต่อกิโลกรัม ในสูตรอาหาร การใช้ แกรมม่าอะมิโนบิวทีริค แอซิดจากข้าวเปลือกงอกในสูตรอาหารไก่กระทงมีผลในทางบวกกับสมรรถนะการผลิต และคุณภาพซากของไก่กระทง ซึ่งระดับที่เหมาะสมของการเสริมสาร แกรมม่าอะมิโนบิวทีริค แอซิด จากข้าวเปลือกงอกควรเสริมในระดับ 2 มีลลิกรัมต่อกิโลกรัม

คำสำคัญ: แกรมม่าอะมิโนบิวทีริคแอซิด ปริมาณการกินได้ การเจริญเติบโต อัตราการเปลี่ยนอาหารเป็นน้ำหนัก คุณภาพซาก ไก่กระทง

TITLE	Effect of Gamma Aminobutyric Acid (GABA) from Germinated Paddy Rice in Ration on Growth Performance and Carcass Characteristics in Broilers
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UNIVERSITY	Mahasarakham University **DATA** 2014

ABSTRACT

This study aims to investigate the effects of GABA levels from germinated paddy rice in ration on growth performance and carcass characteristics in broilers. Two hundred 1- day- old broiler chicks of both sexes were used for 42 days. The chicks were randomly allocated to 20 pens containing 10 chicks each with 4 replicates and assigned to receive one of 5 dietary treatments (inclusion rate 0, 0.5, 1, 1.5 and 2 mg/kg of GABA) in a completely randomized design. The result showed that GABA has been contained in germinated paddy rice was 229.30 mg/kg. Feed intake from week 1 to 4 and week 6 were not significant difference (P>0.05). However, feed intake was significant different (P<0.05) at week 5 in a group 2.0 mg/kg GABA, it was higher than other groups. In addition, the average daily gain in the week 1, 3, 4 and 5 were not significant difference (P>0.05), but highest average daily gain were showed in group of inclusion at 2.0 and 1.0 mg/kg of GABA in week 2 and 6, respectively. Furthermore, feed conversion ratio was not significant different (P>0.05). The eviscerated, breast, drumstick, abdominal fat, wing, liver, spleen, and heart percentage were not significant difference (P>0.05), but the dressing percentage was highest in group of inclusion at 2 mg/kg of GABA, and thigh percentage was highest in group of inclusion at 1 mg/kg of GABA group (P<0.05). Therefore, using GABA from germinated paddy rice in ration was positive effects on growth performance and carcass characteristics in broilers. The optimum level of inclusion rate was 2.00 mg/kg of GABA from germinated paddy rice.

Keywords: GABA, Feed Intake, Average Daily Gain, Feed Conversion Ratio, Carcass Characteristics, Broilers

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CHAPTER 1

Introduction

1.1 Background

Gamma amino butyric acid (GABA), an amino acid that acts as a major inhibitory neurotransmitter in the mammalian central nervous system, is found in high concentrations in the brain area known to be involved in the control of behavior. It inhibits nerve transmission in the brain, calming nervous activity, and plays a role in regulating neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone (DNC, 2012). GABA has been known since 1950 when Eugene Roberts and Jorge Awapara independently discovered that there were prodigious amounts of GABA in the mammalian central nervous system. GABA was virtually undetectable in other tissues. GABA had been long known to exist in plants and bacteria, where its serves as a metabolic role in the krebs cycle (Jorgensen, 2005).

Many researchers reported GABA content increased in rice grain throughout the germinating process,. Moreover, Oh (2003) reported that brown rice germination in chitosan/glutamic acid solution was significant as it increased GABA synthesis activity and the concentration of GABA. GABA content was high (448.79 mg/100g of DM) which was obtained under condition of soaking at 40 ^{o}C for 24 hours and germinating at 40 ^{o}C for 24 hours (Artnaseaw, 2010). Pradeep et al. (2011) also reported soaking and germinating can improve GABA content in millets and legumes. Consequently, the GABA content should increase by germinating seed rice. There are many researchers who have supplemented GABA in experimental diets of animals.

GABA supplementation in a chicken diet is meant to reduce stress, improve growth performance, egg quality and growth in cats (McGuire et al., 1984; Tao et al., 2010). GABA affected meat and carcass quality of broilers (Dai et al., 2012), laying performance, immune activity, and endocrine hormone in broilers (Zheng-sheng, 2009; Zhang et al., 2012). In addition, the effects of GABA should improve erythrocyte, body weight gain and decrease feed conversion ratio in stressed broilers (Chen et al., 2001) GABA increased the level of plasma T3 and growth performance in chickens (Zheng-sheng, 2009; Jianzhen et al., 2010). GABA is very important for animal production because it improves the quality of carcass production, egg quality and moreover reduces stress in the animal production system.

In conclusion, GABA in the diet affects feed intake, feed conversion ratio, average daily gain, and carcass production in broilers. Supplementation of GABA from germinated paddy rice in the diet of broilers is an alternative for animal production. Consequently, this study has selected GABA from germinated paddy rice to supplement the diet of broilers as a feed additive.

1.2 Objectives

1. To study the chemical composition of the germinated paddy rice.
2. To study the effect of GABA levels from germinated paddy rice on the growth performance and carcass characteristics in broilers.
3. To study the effect of GABA levels from germinated paddy rice on hematological values.

1.3 Hypothesis

1. The germinated paddy rice might contain the GABA content.
2. The GABA levels might affect growth performance and carcass characteristics
3. The hematological value in broilers might be significant between the control group and experimental group.

1.4 Scope of Study

This study aims to investigate the effects of GABA levels from germinated paddy rice on growth performance, carcass characteristics, and the blood hematological value in broilers. Two hundred broiler chicks (Arbor Acres) one day old were fed with a basal diet of different levels of GABA 0, 0.5, 1, 1.5 and 2 mg/kg. All 200 chicks were assigned in a completely random design (CRD) that included 5 treatment groups (4 replicates of 10 birds per cage). The recorded data of feed intake, feed conversion ratio, and average daily gain were taken from start until finish of the experiment. Before feeding a diet sample was kept to determine chemical composition, dry matter (DM), crude fiber (CF), crude protein (CP), ether extract (EE), and ash. Blood specimens of thirty five day old chickens were taken to measure the hematological value, total red blood cell, total white blood cell, hematocrit, eosinophils, basophils, monocytes, lymphocytes and heterophils. Forty two day old broilers were randomized and two birds (both sexes) from each group were slaughtered to determine the carcass characteristics, dressing weight, eviscerated weight, drumstick, breast muscle, tight muscle, wing, abdominal fat, spleen, heart and liver.

1.5 Expected Outcomes

1. To know the chemical composition of germinated paddy rice.
2. To know the effect of GABA levels in dietary on feed intake, average daily gain, feed conversion ratio and carcass characteristics in broilers.
3. To know the effects of GABA levels in dietary on hematological values in broilers.

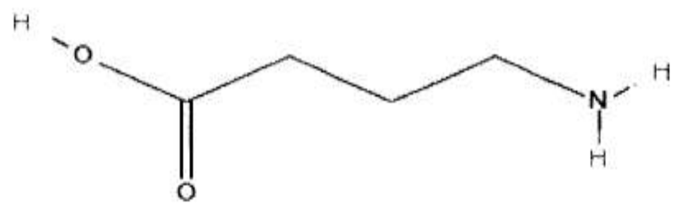
CHAPTER 2

Literature Review

2.1 Gamma Aminobutyric Acid (GABA)

2.1.1 GABA structure

GABA, a four carbon non protein amino acid, is a significant component of the free amino acid pool. GABA has an amino group on the γ-carbon rather than on the α-carbon, and exists in an unbound form and high solution in water (Shelp et al., 1999). The structure of GABA has an amino group (NH_2) and a carboxyl group (COOH) (Figure 2.1).



Source: Yoshikuni, (2008)

Figure 2.1 Chemical structure of gamma amino butyric acid (GABA)

2.1.2 Source of GABA

GABA has been long known to exist in plants and bacteria (Jorgensen, 2005), and it can convert from bacteria to plants and vertebrates (Bouche et al., 2004). In animals, GABA is distributed in non-neural tissue including the peripheral nervous and endocrine system (Tillakaratne et al., 1995). It is found in high concentrations in brain areas known to be involved to the control of digestive behavior (Jonaidi et al., 2002) . For plants, GABA increased 20% of CO_2 in strawberry fruit while in storage (Deewatthanawong et al., 2010) and soaking brown rice at a temperature of 40^oC for 24 hrs and germinating at a temperature of 40^oC for 24 hrs (Artnaseaw, 2010). Thus

GABA concentration increased several fold in response to many diverse stimuli, including heat shock, mechanical stimulation, hypoxia and phytohormones (Shelp et al., 1999). In conclusion, GABA accumulated in many organisms from prokaryote to eukaryote and at various levels concentration. Notably, GABA content increased in rice seed by germinated processing.

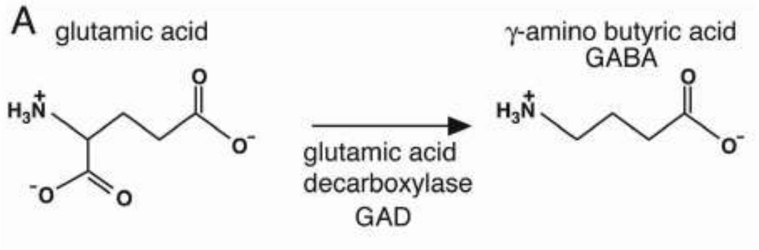
2.1.3 GABA function

GABA is one of the major inhibitory neuro-transmitters in the central nervous system in mammalian and bird brain. It plays the role of an exciter in the nervous system. Many neurological disorders, such as seizures, Parkison's disease, Stiff-man syndrome, and schizophrenia are related to alterations of GABA levels in the brain (Bao et al., 1995). The direct excitement effect to some regions of the brain including the hypothalamus makes it important to consider, mechanism of feed intake, growth rate, and feed conversion ratio regulation in broilers (Jonaidi et al., 2002), reduced stress in broiler (Dai et al., 2012), and improved immune function of broiler chickens (Zheng-sheng, 2009). Hence, the function of GABA is to reduce stress, improve immune function and growth performance in broiler chickens and others.

2.2 Metabolism of GABA in Broilers

GABA has been used as a feed additive in animal dietary (Puia et al., 2011; Dai et al., 2012) and also in humans (Zafra et al., 1991; Khaszali, 2002). With respect to rice (Artnaseaw, 2010), strawberry (Deewatthanawong et al., 2010) and other fruits, several researches added pure GABA in the diet and injected GABA in chickens (Usami et al., 1989; Kryukov et al., 1990; Denbow, 1991; Chen et al., 2001; Jonaidi et al., 2002; Zheng-sheng, 2009; Jianzhen et al., 2010; Dai et al., 2011; Dai et al., 2012; Babapor et al., 2012; Zhang et al., 2012; Pereira et al., 2013).

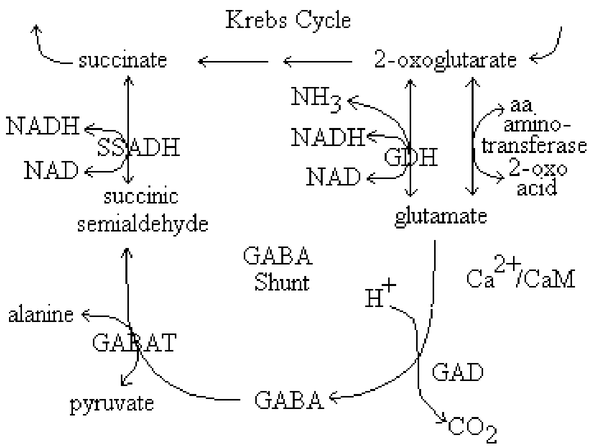
The pathway converts glutamate to succinate via GABA and is called the GABA shunt (Shelp et al., 1999). In addition, GABA was synthesized from glutamate by the enzyme glutamic acid decarboxylase (GAD) (Kaplan, 2005) (Figure 2.2).



Source: Kaplan, (2005)

Figure 2.2 GABA is synthesizes from glutamate by the enzyme glutamic acid decarboxylase

Glutamine, synthesized exclusively in astrocytes, is quantitatively the most significant precursor for the excitatory and inhibitory neurotransmitters, glutamate and GABA (γ-aminobutyrate), respectively. Glutamate subsequent to its release and receptor interaction is taken up by surrounding cellular elements, predominantly astrocytes. In astrocytes, glutamate is directly converted to glutamine, or it enters the tricarboxylic acid (TCA) cycle. Glutamine is transferred to glutamatergic neurons to serve as precursor for glutamate or for TCA cycle intermediates. The latter is important due to the lack of anaplerosis in neurons. GABA is also taken up by surrounding cellular elements following receptor interaction. However, neuronal reuptake seems to be most important in case of GABA. GABA is either reutilized as neuro-transmitter or metabolized via the GABA shunt. To the extent that GABA is lost to the astrocytic compartment, glutamine serves as the precursor for synthesis. This occurs by deamidation to glutamate and further decarboxylation to GABA. GABA is formed in *vivo* via a metabolic pathway called the GABA shunt. The initial step in this pathway utilizes ketoglutarate formed from glucose metabolism via the Krebs cycle. Ketoglutarate is then transaminated by oxoglutarate transaminase (GABA-T) to form glutamate, the immediate precursor of GABA. Finally, glutamate is decarboxylated to form GABA by the enzyme(s) glutamic acid decarboxylase (GAD). GAD is expressed only in GABAergic neurons and in certain peripheral tissues which are also known to synthesize GABA. Like most neurotransmitters, GABA is stored in synaptic vesicles and is released in a Ca^{2+} dependent manner upon depolarization of the presynaptic membrane. Following release into synaptic cleft, GABA's actions are terminated principally by reuptake into presynaptic terminals and/or surrounding glia. GABA is also metabolized by GABA-T to form succinic semialdehyde. This transamination will regenerate glutamate when it occurs in the presence of ketoglutarate. Succinic semialdehyde is oxidized by succinic semialdehyde dehydrogenase (SSADH) to succinic acid which then reenters the Krebs cycle (Paul, 2000)



Source: David, (2009)

Figure 2.3 Krebs cycle of GABA

2.3 Germinated Paddy Rice

Rice accounts for over 22% of global energy intake. More than 90% of the world's rice is grown and consumed in Asia (Roohinejad et al., 2009). Brown rice contains more nutritional components, such as dietary fiber, phytic acid, vitamin E, vitamin B, and gamma amino butyric acid (GABA) than ordinary milled rice (Banchuen et al., 2009). Furthermore, germination is the most common and effective process to improve the quality of cereal gain. Soaking is the first step in water penetration; which transforms the active tissue into living tissue. In this step, the gain's metabolism is activated in preparation for germination. During germination, the gain nutrient reserves degrade and are used for respiration and the synthesis of new cells that form the developing embryo, causing changes in the nutritional and biochemical composition (Maisont et al., 2010). Oh, (2003) found that the GABA concentration was enhanced in the germinated brown rice when compared to non-germinated brown rice. GABA content in brown rice was 17.87mg/100g of dry matter after incubated 24hrs (Karladee et al., 2012). Paddy rice was soaked about 33% moisture and then germinated in an incubator (28-30% of moiture) for 12-60 hrs the GABA content increased from 80 to 220mg/100g embryo fresh weight (Maisont et al., 2010). On the other hand, highest GABA content was obtained when brown rice soaked in a solution of citric acid with pH = 3 at 30±2 ^{o}C germinated in a closed vessel for 36 hrs for Sangyod Phatthalung and Chaing Phatthalung and 48 hrs for Niaw Dam Peuak Dam rice (Banchuen et al., 2010). However, several researchers studied the flow rate of water on the soaked paddy rice. The highest GABA content in germinated paddy rice was obtained when the water flowed 3 l/min/kg (Pilawut et al., 2012). High GABA content (24.9mg/100g) after soaking for 3 hrs and gaseous treatment for 21 hrs at 35 ^{o}C (Komasuzaki et al., 2007). Pre-germinated brown rice was prepared by soaking in water for 72 h at 30 ^{o}C followed by drying to 13–15% moisture content at 15 ^{o}C in a low-humidity artificial weather-controlled room. Total dietary fiber, total ferulic acid and GABA contents of the pre-germinated brown rice were higher than those of ordinary brown rice or polished rice (Ohtsubo et al., 2005). Zhenxin et al. (2006) reported that glutamate decarboxylase activities in germinating brown rice were consistently higher than those in paddy-rice in the process of the germination and storage. Germinating on wet cotton cloth for 24-60 hrs provided free GABA significantly higher than soaking in water ($p \leq 0.05$). Germination time increased to 96 h, and soaking in water provided free GABA content significantly higher than germinating on wet cotton cloth (Sovaphan et al., 2011). The best condition for the content of GABA in brown rice was when the temperature of seed soaking was 35 ^{o}C at 24 hrs and the temperature of seed germination was 30 ^{o}C at 20 hrs (Su-hong, 2011). In conclusion, all methods based on the reaction in the bio-function change of the seed during soaking and germination depends on the time and temperature. Particularly, the Artnasseaw’s method (2010) proved to be more interesting than other methods, as presented in Table 2.1 and Table 2.2.

Table 2.1 GABA content in rice seed under soaking process, various temperature and the soaking time

Temperature (^oC)	Soaking time (hrs)	GABA content (mg/kg)
	5	26.60 ± 0.10^a
30	12	99.64 ± 0.12^c
	24	258.05 ± 0.08^f
	5	164.06 ± 0.39^d
40	12	254.03 ± 0.02^f
	24	293.63 ± 0.02^h
	5	90.18 ± 0.21^b
50	12	175.57 ± 0.11^e
	24	280.57 ± 0.03^g

Source: Artnasseaw, (2010)

Table 2.2 GABA content in rice seed under germinating process, various temperature and the germinating time

Temperature (^oC)	Germinating time (hrs)	GABA content (mg/100g)
	12	86.10 ± 1.87^a
30	24	215.00 ± 0.60^b
	36	202.74 ± 2.45^c
	12	259.54 ± 4.06^d
40	24	448.79 ± 2.15^e
	36	445.29 ± 1.25^e
	12	171.45 ± 2.29^f
50	24	197.40 ± 1.5^c
	36	112.96 ± 1.14^g

Source: Artnasseaw, (2010)

Table 2.3 GABA content of germinated brown rice at various soaking solution

Soaking solution	GABA content (mg/100g)
Citrate buffer, pH 3.0	8.36 ± 0.06[d]
Citrate buffer, pH 5.0	5.79 ± 0.10[b]
Phosphate buffer, pH 7.0	4.58 ± 0.31[a]
Distilled water	6.36 ± 0.06[c]

Source: Banchuen et al. (2009)

GABA content in germinated brown rice was higher than un-germinated, as shown in table 2.4, because during germination the grain nutrient reserve degraded and were used for respiration and the synthesis of new cells that form the developing embryo (changes in the nutritional and biochemical composition) (Changyu et al., 2011).

Table 2.4 Chemical composition of un-germinated and germinated brown rice

Composition (%)	Un-germinated brown rice	Germinated brown rice
GABA	2.64 ± 0.11[a]	44.53 ± 1.93[b]
Protein	8.93 ± 0.04[a]	9.20 ± 0.04[b]
Fat	2.24 ± 0.02[a]	2.74 ± 0.03[b]
Ash	1.20 ± 0.01[a]	1.50 ± 0.07[b]
Total dietary fiber	4.13 ± 0.28[a]	5.26 ± 0.28[b]
Total free sugar	0.50 ± 0.02[a]	1.00 ± 0.01[b]
Starch	75.72±0.77[a]	73.97±0.22[b]

Source: Banchuen et al. (2009) and Maisont et al. (2010)

2.4 Broiler Chickens

The term broiler is applied to chicks that have been especially bred for rapid growth. Broiler strains are based on hybrid crosses between Cornish White, New Hampshire and White Plymouth Rock. Chickens of broiler strains have been selected for rapid weight gain and efficient utilization of feed. Broilers are usually allowed to feed on an *ad libitum* basis to ensure rapid development to market size, although some interest has been expressed in controlling feed intake in an attempt to minimize the development of excessive carcass fat. Broilers are marketed at a wide range of ages and body weights. Females may be grown to 900- to 1,000g body weight to supply Cornish hens, mixed sexes may be reared to 1.8 to 2 kg for use as whole birds and specialty parts, and males may be grown to 2.8 to 3 kg for deboned meat. Thus it is difficult to establish a single set of requirements that is appropriate to all types of broiler production. Furthermore, nutrient requirements may vary according to the criterion of adequacy. In the instance of essential amino acids, greater dietary concentrations may be required to optimize efficiency of feed utilization than would be needed to maximize weight gain. There is evidence that the dietary requirement for lysine to maximize yields of breast meat of broilers is greater than that needed to maximize weight gain (NRC, 1994).

Source: Barry, (2014)

Figure 2.4 Broiler chicken

2.5 Nutrient Requirements for Broiler

Expression of a requirement for any nutrient is relative, and many factors must be considered. Many nutrients are interdependent, and it is difficult to express requirements for one without consideration of the quantity of the other. Examples include the relationships that exist between lysine and arginine and among calcium, phosphorus, and vitamin D3 levels in the diet. Other factors that may affect requirements include age and gender of the animal. Some studies suggest that males require greater quantities of nutrients than do females at a similar age; however, when expressed as a percentage of the diet, there seems to be little difference in nutrient requirements of the sexes. The requirements for many nutrients seem to diminish with age, but for most nutrients there have been few research studies designed to precisely estimate requirements for all age periods, especially for those beyond 3 weeks of age. On the other hand, several expressions of nutrient requirements can be only a guideline representing a consensus of research reports. These guidelines must be adjusted as necessary to fit the wide variety of ages, sexes, and strains of broiler chickens.

Table 2.5 Typical body weights, feed requirements, and energy consumption of broilers

Age (weeks)	Body Weight (g)		Weekly Feed Consumption (g)		Cumulative Feed Consumption (g)		Weekly Energy Consumption (kcal *ME*/bird)		Cumulative Energy Consumption (kcal *ME*/bird)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	152	144	135	131	135	131	432	419	432	419
2	376	344	290	273	425	404	928	874	1,360	1,293
3	686	617	487	444	912	848	1,558	1,422	2,918	2,715
4	1,085	965	704	642	1,616	1,490	2,256	2,056	5,174	4,771
5	1,576	1,344	960	738	2,576	2,228	3,075	2,519	8,249	7,290
6	2,088	1,741	1,141	1,001	3,717	3,229	3,651	3,045	11,900	10,335
7	2,590	2,134	1,281	1,081	4,998	4,310	4,102	3,459	16,002	13,794
8	3,077	2,506	1,432	1,165	6,430	5,475	4,585	3,728	20,587	17,522
9	3,551	2,842	1,577	1,246	8,007	6,721	5,049	3,986	25,636	21,508

NOTE: Values are typical for broilers fed well-balanced diets providing 3,200 kcal *ME*/kg.
Source: NRC. (1994)

The values given in Table 2-6 are generally minimum levels that satisfy general productive activities and (or) prevent deficiency syndromes. Requirements are presented for specific age periods. These age periods are based on the chronology for which research data were available. These nutrient requirements are often implemented for younger age intervals or on a weight-of-feed consumed basis.

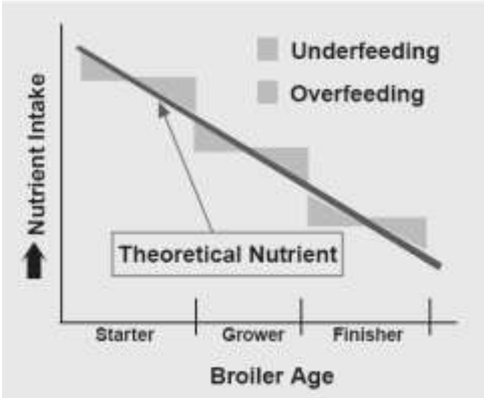
Table 2.6 Nutrient requirements of broilers as percentages or units per kilogram of diet (90 percent dry matter)

Nutrients	Unit	0-3 weeks(3,200)	3-6 weeks (3,200)	6-8weeks(3,200)
Protein and amino acid				
Crude protein	%	23.00	20.00	18.00
Arginine	%	1.25	1.10	1.00
Glycine+serine	%	1.25	1.14	0.97
Histidine	%	0.35	0.32	0.27
Isoleucine	%	0.80	0.73	0.62
Leucine	%	1.20	1.09	0.93
Lycine	%	1.10	1.00	0.55
Methionine	%	0.50	0.38	0.32
Methionine+cystine	%	0.90	0.72	0.60
Phenylalanine	%	0.72	0.65	0.56
Phenylalanine+tyrosine	%	1.34	1.22	1.04

Table 2.6 Nutrient requirements of broilers as percentages or units per kilogram of diet (90 percent dry matter) (continue)

Nutrients	Unit	0-3 weeks(3,200)	3-6 weeks (3,200)	6-8weeks(3,200)
proline	%	0.60	0.55	0.46
threonine	%	0.80	0.74	0.68
tryptophan	%	0.20	0.18	0.16
valine	%	0.90	0.82	0.70
Fat				
Linoleic acid	%	1.00	1.00	1.00
Macrominerals				
calcium	%	1.00	0.90	0.90
chlorine	%	0.20	0.15	0.12
Magnesium	mg	600	600	600
Nonphytase phosphorus	%	0.45	0.35	0.30
potasium	%	0.30	0.30	0.30
sodium	%	0.20	0.15	0.12
Trace minerals				
copper	mg	8	8	8
Iodine	mg	0.35	0.35	0.35
Iron	mg	80	80	80
Manganese	mg	60	60	60
Selenium	mg	0.15	0.15	0.15
Zinc	mg	40	40	40
Fat soluble vitamins				
A	IU	1,500	1,500	1,500
D3	ICU	200	200	200
E	IU	10	10	10
K	mg	0.50	0.50	0.50
Water soluble vitamins				
B12	mg	0.01	0.01	0.007
Biotin	mg	0.15	0.15	0.12
Choline	mg	1,300	1,000	750
felacin	mg	0.55	0.55	0.50
Niacin	mg	35	30	25
Phantothenic acid	mg	10	10	10
Pyridonic	mg	3.5	3.5	3.0
Riboflavin	mg	3.6	3.6	3.0
Thiamin	mg	1.80	1.80	1.80

Source: NRC. (1994)



Source: FAO, (2010)

Figure 2.5 Nutrient intake and broiler age

If producers initially feed exactly at the bird's requirement, before long they are feeding above the bird's need and expensive nutrients are wasted. Conversely, if birds are fed below their nutrient requirement till they "grow into" the ration, then rations are deficient for a period of time (Figure 2.5) and performance can wane (FAO, 2010).

2.6 Effect of GABA on Poultry

GABA, a major inhibitory neuro-transmitter of mammalian and bird's central nervous system (Babapour et al., 2012), is found in a wide range of organisms, from prokaryote to vertebrates (Tillakaratne et al., 1995). They had known that GABA might reduce stress in humans and animals, though some researchers applied it to increase the quality of poultry feeding products according with Dai et al. (2012).

2.6.1 Effect of GABA on feed intake

Feeding birds are controlled by centers in the hypothalamus, situated beneath the cerebrum in the brain. It was originally proposed that there were two centers of activity. The first of these was the feeding center (lateral hypothalamus), which caused the animal to eat food unless inhibited by the second, the satiety center (ventromedial hypothalamus), which received signals from the body as a result of consumption of food. Quite simply it was considered that the animal would continue to eat unless the satiety center received signals which inhibited the activity of the feeding center. There is little doubt that this is an over simplification and, although the hypothalamus does play an important role in intake regulation (McDonald, 2002). In addition, Denbow (1991) and Jonaidi et al. (2002) studied the effect of GABA on feed intake in broilers, and reported that GABA affected feed intake in broilers. Xie et al. (2012) also reported that feed intake was significantly increased when supplemented with 80 mg/kg GABA in the basal diet chickens under heat stress. Dai et al. (2011), who studied the effects of dietary glutamate and GABA on performance, carcass characteristics and serum parameters in broilers under circular heat stress, reported that the GABA effect on feed intake significantly increased (see Table 2.7). Moreover, Jing et la. (2010) and Tan et al. (2012) reported that GABA can improve feed intake, reproduction and anti stress. These reasons are due to GABA affects on the central nervous system, the brain collecting information from special senses and receptors in the digestive tract wall, and metabolizing tissues. This information is integrated and used to determine what food to eat. In contrast, Tao et al. (2010) who studied effects and mechanism of GABA on laying performance and egg quality of laying hens under high temperature in summer also reported feed intake was not significantly affected by supplemented with 25,50,75,100 mg/kg GABA in basal diet. It’s because the GABA levels did not have enough affect on the animals because those studies were conducted under heat stress temperatures. Ootherwise, D’Mello (2000) reported that if animals are already well fed and managed so that the rate of production of meat milk or eggs is already optimal, then to stimulate intake would only lead to more deposition of fat, which is not required in modern animal production. However, GABA had been affected.

2.6.2 Effect of GABA on average daily gain

The average daily gain was affected by GABA as reported by Chen et al. (2001) who studied effect of GABA on the heat stress in broilers and reported that average daily gain was significantly increased. In addition, Zou et al. (2009) supplemented GABA in the basal diet of growth finishing swine under heat stress, and reported that the average daily gain was significantly increased. Jaicheng et al. (2008) reported that supplemented GABA in the basal diet can increase average daily gain in pigs. Zhang et al. (2012), who studied effect of dietary GABA in laying performance, egg quality, immune activity and endocrine hormone in heat stress Roman hens, reported that egg production increased. Xie et al. (2012) also reported that average daily gain significantly increased by supplemented 80 mg/kg GABA in the basal diet of chickens under heat stress. As a result, many reports showed GABA affected average daily gain in animals under heat stress. It is due to increasing the feed intake and otherwise the central nervous system control of all the secreting hormones in the body involving the thyroid hormones (T3 and T4). The thyroid hormone is related to metabolizing in the body.

2.6.3 Effect of GABA on feed conversion ratio

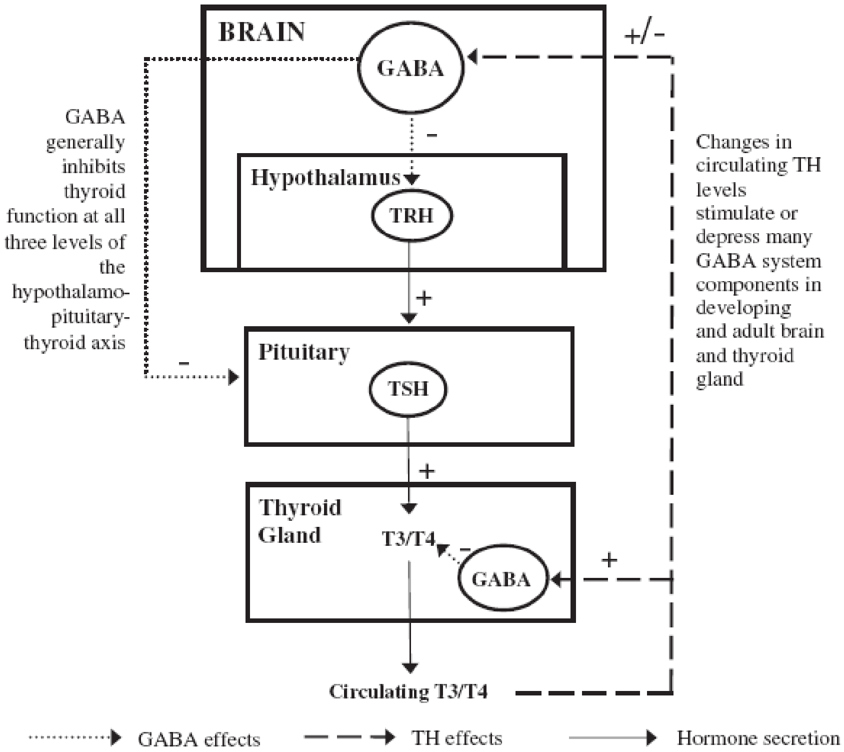
Feed conversion ratio had been shown as reported by Chen et al. (2001), who studied the effect of GABA on the heat stress broilers, and reported that the feed conversion ratio significantly decreased. Zhang et al. (2012), who studied the effects of dietary GABA in laying performance, egg quality, immune activity, and endocrine hormone in heat stress Roman hens, reported that feed egg ratio increased. ZOU et al., (2009) supplemented 10 mg/kg of GABA in the basal diet of growth finishing swine in hot simmer, reported that feed conversion ratio increased. Dai et al. (2011) who studied the effects of dietary glutamate and GABA on performance, carcass characteristics and serum parameters in broilers under circular heat stress, reported that GABA affected the feed conversion ratio as significantly increasing. In contrast, Zhigang et al. (2013), who studied effectd of GABA on performance parameters and some plasma metabolites in cherry valley duck under high ambient temperature, reported that the feed conversion ratio decreased. Consequently, the feed conversion ratio was affected by adding GABA in the diet of animals.

2.6.4 Effect of GABA on carcass characteristics

Carcass characteristics reported by Dai et al. (2011) showed the effects of dietary GABA on performance carcass characteristics and serum parameters in broilers under circular heat stress included increased eviscerated carcass breast muscle and abdominal fat percentage (see Table 2.8). Dai et al. (2012) also reported that GABA effected on carcass yield in broilers under the heat stress. Moreover, Xiao-ting et al. (2009) reported that GABA effected carcass characteristics and enhanced thyroid function in growth finishing swine. Shichang et al. (2007) reported that GABA affected thyroid hormone decrease and increased in a concentrate of glucose and corticosteroid in broilers. Wang et al. (2011) found that the combination of vitamin E and GABA could significantly decrease the myofiber diameter of broilers in 42 days and then improve breast meat quality. In conclusion, the GABA possibly affected carcass characteristics in poultry.

2.6.5 Effect of GABA on hematological value

GABA, a major inhibitory neurotransmitter of mammalian and bird's central nervous system (Babapour et al., 2012), is found in a wide range of organisms, from prokaryote to vertebrates (Tillakaratne et al., 1995). The GABA functions in thyroid gland to produced thyroid hormones, T3 and T4 (Wiens et al., 2006) are presented in Figure 2.6. Its hormones, T3 and T4 are involved in a wide range of metabolic activities influencing the growth and development of organisms (Arhiv et al., 2000). In addition, a diet added with GABA improved the immunity function of broilers (Zhen-sheng et al., 2009). On the other hand, Chen et al. (2010) reported that 30 mg/kg GABA in diet could reduce the level of T4 and increase the level of T3. Consequently, GABA affected hematological value in poultry.



Source: Wiens et al. (2006)

Figure 2.6 GABA and thyroid hormone secreting

Table 2.7 Hematological value of broilers

Items	Min-Max	Mean
Total red blood cells (x10^6 cell/µl)	2.00-3.00	2.26
Hemoglobin (g/dl)	8.00-10.00	8.89
Packed cell volume (%)	28.00-37.00	32.18
Total white blood cells (cell/µl)	16,000-25,000	20,400
Eosinophils (%)	2.00-9.00	5.83
Basophils (%)	1.00-5.00	2.65
Monocytes (%)	1.00-7.00	4.20
Lymphocytes (%)	54.00-73.00	63.68
Heterophils (%)	16.00-31.00	23.70

Source: Nowaczewski et al. (2012)

CHAPTER 3

Research Methodology

3.1 Preparing Germination Paddy Rice

Jasmine rice was chosen for this study. It was weighed before soaking in clean water at a temperature 40^{o}C for 24 hrs and germination at temperature 40^{o}C for 24 hrs. The germinated paddy rice was dried under sunlight conditions for thorough drying. The dried germinated paddy rice was milled and mashed. The mashed rice was analyzed with HPLC (High Performance Liquid Chromatography) and proximate analysis as described by AOAC (1998).

3.2 Animals

A total of 200 broiler chicks (Arbor Acers) 1 day old were used in this study. This study was divided into 5 treatments (4 replicates, 10 chicks each). All chickens were provided with a basal diet supplemented with GABA levels from germinated rice at 0, 0.5, 1, 1.5, and 2 mg/kg of diet. All chicks were fed in evaporated cooling system cages with controlled temperature, a 100 watt lamp, and rice husk as litter. The cages were divided into 20 pens, 10 chicks in each pen the first 14 day old chicks were brooded with a heat lamp. The brooder temperature was maintained at around 35 ^{o}C up to 7 days and gradually decreased to 29 ^{o}C, and later the chickens were kept at room temperature. The experimental chicks were fed broiler starter from one-day to three weeks old and broiler finisher rations in the fourth to sixth week *ad libitum* (NRC, 1994). The birds had free access to feed and clean drinking water. All chicks were vaccinated against new castle disease and infectious bronchitis disease.

3.3 Dietary

The diet was a basal diet based on NRC (1994) with GABA added levels from germinated paddy rice. Levels of 0, 0.5, 1, 1.5, and 2 mg/kg of diet represented treatment T1, T2, T3, T4 and T5 respectively. The basal diet used corn meal, soybean meal 44% of CP, full fat soybean, soybean oil, methionine, mon-calciumphosphat, salt, limestone, and vitamin premix. These are presented in Table 3.1. The diet was formulated one week before feeding to make sure that it did not oxidize oil with oxygen, lose some nutrients, or become rancid and reduce feed intake in the chickens.

Table 3.1 Ingredients composition (%) of broiler starter and finisher diets different levels of GABA

Ingredients	Level of GABA*, mg/kg				
	0.00	0.50	1.0	1.5	2.0
Broiler starter					
Yellow maize	44.50	44.50	44.50	44.50	44.50
Soybean oil	3.60	3.60	3.60	3.60	3.60
Soybean meal (44 %CP)	32.40	32.40	32.40	32.40	32.40
Full fat soybean	14.80	14.80	14.80	14.80	14.80
Monocalcium phosphate	2.20	2.20	2.20	2.20	2.20
Limestone	1.60	1.60	1.60	1.60	1.60
Vitamin premix	0.20	0.20	0.20	0.20	0.20
DL-Methionine	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30
Calcium, %	0.97	0.97	0.97	0.97	0.97
Phosphorus, %	0.60	0.60	0.60	0.60	0.60
Lysine#	1.18	1.18	1.18	1.18	1.18
Methionine#	0.55	0.55	0.55	0.55	0.55
Tryptophan#	0.34	0.34	0.34	0.34	0.34
Treonine#	0.89	0.89	0.89	0.89	0.89
ME (kcal/kg) #	3219.09	3219.09	3219.09	3219.09	3219.09
Germinated paddy rice (g/kg)	0.00	2.18	4.36	6.54	8.72
Broiler finisher					
Yellow maize	54.70	54.70	54.70	54.70	54.70
Soybean oil	2.60	2.60	2.60	2.60	2.60
Soybean meal (44 %CP)	26.40	26.40	26.40	26.40	26.40
Full fat soybean	11.90	11.90	11.90	11.90	11.90
Monocalcium phosphate	2.20	2.20	2.20	2.20	2.20
Limestone	1.40	1.40	1.40	1.40	1.40

Table 3.1 (continued)

Ingredients	Level of GABA*, mg/kg				
	0.00	0.50	1.0	1.5	2.0
Vitamin premix	0.20	0.20	0.20	0.20	0.20
DL-Methionine	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Calcium, %	0.88	0.88	0.88	0.88	0.88
Phosphorus,%	0.58	0.58	0.58	0.58	0.58
Lysine[#]	1.00	1.00	1.00	1.00	1.00
Methionine [#]	1.42	1.42	1.42	1.42	1.42
Tryptophan[#]	0.28	0.28	0.28	0.28	0.28
Threonine[#]	0.77	0.77	0.77	0.77	0.77
ME (kcal/kg) [#]	3212.07	3212.07	3212.07	3212.07	3212.07
Germinated paddy rice (g/kg)	0.00	2.18	4.36	6.54	8.72

* GABA contents in germinated paddy rice is 229.30 mg/kg

Calculated value

Table 3.2 The price of feedstuffs during February until March, 2014

Feedstuffs	Unit price (Baht/kg)
Yellow maize	12
Soybean meal (44% CP)	20.71
Full fat soybean (36% CP)	28.4
Soybean oil	55
Methionine	160
Monocalciumphosphate	30
Limestone	2
Salt	7
Vitamin premix	88.5

3.4 The Chicken Feeding

This study was randomly assigned and divided into five treatment groups (4 replicates of 10 chicks per cage). Each group received two phases of diet. The first phase (starter) was 1-21 days old, and the second phase (finisher) was 22-42 days old (NRC, 1994). The chicks were fed on rice husk spread on all spaces of the cage. Chicks brooded with a 100 watt lamp for 14 days. When the chicks were 1 week old, they were vaccinated against new castle disease and infectious bronchitis. They were fed *ad libitum* and provided with clean water and diet two times a day, morning and evening over the experimental duration. All treatments received GABA diet at the different levels of, 0, 1.5, 1, 1.5, and 2 mg/kg. Chickens and feed were checked every day in the morning. Weighing of chickens was done weekly. When the chickens were 35 days old blood was taken to count the total red blood cells, total white blood cells and other cells typical of white blood cells.

On the 42nd days two chickens, male and female in each group, were slaughtered for measuring carcass characteristics, dressing, eviscerated, breast, drumstick, thighs, abdominal fat, wing, gizzard, liver, spleen, and heart percentage.

3.5 Data Collection

This study investigated growth performance, carcass characteristics and hematological value in broilers.

3.5.1 The growth performance

For growth performance, there were average daily gains (ADG), feed intake (FI), and feed conversion ratio (FCR), that were calculated by the formula below.

Feed intake (FI) is a parameter that formulates from provided feed weight and remained feed weight every week.

$$\text{FI (g/h/d)} = \frac{\text{total feed weight – remained feed weigh}}{\text{number of broilers}}$$

Average daily gain (ADG) was formulated from initial weight with finished weight every week.

$$\text{ADG} = \frac{\text{finished body weight – initial body weight}}{\text{number of days}}$$

Feed conversion ratio (FCR) was formulated from FI and ADG.

$$\text{FCR} = \frac{\text{FI (g/h/d)}}{\text{ADG (g/h/d)}}$$

The mortality rate was formulated by total mortality amount with total chickens.

$$\text{Mortality rate} = \frac{\text{total of mortality amount x 100}}{\text{total of broilers}}$$

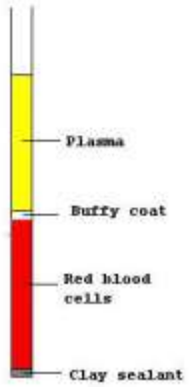
Production index (PI)

$$\text{PI} = \frac{\text{survival broilers (\%) x body weight gain (kg) x 100}}{\text{number of days x FCR}}$$

3.5.2 Hematological value

Blood specimens were collected at 35 days old for hematological studies. Two ml of blood was collected from the wing vein of every bird by puncturing with a disposable syringe and kept in a sterile test tube containing anticoagulant (ADTA) at a ratio of 1:10. The hematological studies were performed within a day of blood collection. Hematological parameters were total red blood cells count (TRC), total white blood cells count (TWC), packed cell volume (PCV) and separated white blood cells count.

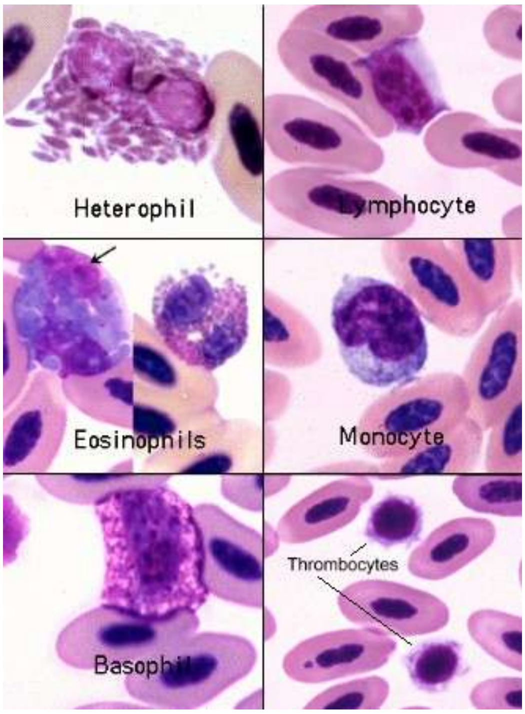
Pack cell volume (PCV) used the capillary tube 75 cm of length and filled the blood 2/3 of tube close it with plasticine. After that it was taken to a hematocrit centrifuge at 2500 rpm speed for 5 minutes and then the PCV scale was read,



Source: Durham (2014)

Figgue 3.1 The sample of blood after taken from hematocrit centrifuge in tube

Smearing the blood by dropping the blood on the slide and smearing left it dry in 3 minutes. One to two drops of buffer were added on the slide, and then more Giem-Wright's strain was added and left for 3 minutes. After, it was cleaned with flowing water to make sure it was clean before being left to dry. The typical white blood cells were looked at under a microscope. (See fig. 3.2)



Source: Meshell, (2014)

Figgue 3.2 The typical white blood cell

3.5.3 Carcass characteristics

The 42 day old chickens were slaughtered and weighed. All the parts, such as live weight, dressed carcass, eviscerated carcass, breast, thigh, drumstick, abdominal fat, spleen, liver, gizzard, and heart were evaluated.

Dressed carcass percentage (DC %)

$$DC (\%) = \frac{\text{dressed weigh x 100}}{\text{live weigh}}$$

Eviscerated carcass percentage (EC %)

$$EC (\%) = \frac{\text{eviscerated weigh x 100}}{\text{live weigh}}$$

Wing percentage (W %)

$$W (\%) = \frac{\text{wing weigh x 100}}{\text{eviscerated weigh}}$$

Thigh percentage (T %)

$$T (\%) = \frac{\text{thigh weigh x 100}}{\text{eviscerated weigh}}$$

Breast percentage (B %)

$$B (\%) = \frac{\text{breast weigh x 100}}{\text{eviscerated weigh}}$$

Drumstick percentage (D %)

$$D (\%) = \frac{\text{drumstick weigh x 100}}{\text{eviscerated weigh}}$$

Abdominal fat percentage (AF %)

$$AF (\%) = \frac{\text{abdominal fat weigh x 100}}{\text{live weigh}}$$

Spleen percentage (S %)

$$S (\%) = \frac{\text{spleen weigh x 100}}{\text{live weigh}}$$

Liver percentage (L %)

$$L (\%) = \frac{\text{liver weigh x 100}}{\text{live weigh}}$$

Gizzard percentage 、

$$G (\%) = \frac{\text{gizzard weigh x 100}}{\text{live weigh}}$$

Heart percentage (H %)

$$H (\%) = \frac{\text{heart weigh x 100}}{\text{live weigh}}$$

3.5.4 Economic benefit return

Feed cost per gain (FCG)= feed cost (FC)/survival

Salable bird return (SBR)= price of live chicken (45 baht)x BW

Net profits return per bird (NPR) = SBR – FCG

Return of investment (ROI) by comparing experiment with control

3.6 Statistical Analysis

Analysis of variance (ANOVA) by randomly complete design (RCD) and comparison of the variance mean by Duncan’s new Multiple Range Test (DMRT) using the statistical analysis system (SAS, 1998). The level of significance was determined at P<0.05.

CHAPTER 4

Results and Discussion

4.1 Chemical Composition of Diets

The chemical composition of the diet was analyzed by proximate analysis. The results showing moisture, ash, crude fiber, crude protein, and ether extracts are presented in Table 4.1, As a result, chemical composition of both phases was similar as that reported by the NRC (1994). The chemical composition percentage of diet starter and finisher are presented in Table 4.2. The GABA content in germinated paddy rice was 229.30 mg/kg and was analyzed by high performance liquid chromatography.

Table 4.1 The components of germinated and un-germinated paddy rice

Components	Paddy rice	
	Un-germinated	Germinated
DM (%)	98.18	94.3
Ash (%)	5.48	3.76
CP (%)	6.40	6.86
CF (%)	14.08	8.83
EE (%)	4.11	3.96

Table 4.2 Nutrients composition of broiler starter and finisher diets with different levels of GABA

Nutrient composition	Level of GABA, mg/kg				
	0	0.5	1.0	1.5	2.0
Broiler starter					
Dry matter, %	91.57	91.75	91.85	91.83	91.83
Ash, %	7.37	7.70	7.55	7.77	7.87
Crude protein, %	25.27	24.61	24.60	25.06	23.94
Crude fiber, %	2.88	3.45	3.24	3.09	3.28
Ether extract, %	8.14	8.17	8.31	8.54	9.02
Broiler finisher					
Dry matter, %	92.44	92.59	92.55	92.58	92.62
Ash, %	7.15	6.96	7.24	7.15	6.33
Crude protein, %	21.92	20.70	21.20	20.49	19.77
Crude fiber, %	2.18	2.50	2.78	2.94	2.37
Ether extract, %	6.72	6.71	7.51	6.22	5.99

4.2 Effect of GABA Levels on Feed intake in Broilers

The effect of GABA from germinated paddy rice in the ration of feed intake is presented in Table 4.3. Based on the results, the effect of GABA levels on feed intake in the week 1, 2, 3, 4, and 6 was not significantly different (P>0.05), while the 5th week was significantly different (P<0.05). Feed intake levels were 163.27, 164.89, 137.16, 159.69, and 189.88 g/h/d in the treatment groups that were supplemented with levels of GABA 0, 0.5, 1, 1.5 and 2 mg/kg of diet, respectively. These results showed that the supplemented GABA level of 2 mg/kg affected feed intake higher (189.88^{a} g/h/d) than other treatment groups. These results were according to Denbow (1991) and Jonaidi et al. (2002), who studied the effect of GABA on the feed intake in broilers, and reported that GABA affected feed intake in broilers. Xie et al. (2012) also reported that feed intake significantly increased by supplementing with 80 mg/kg GABA in the basal diet chickens under heat stress. Dai et al. (2011) studied effects of dietary glutamate and GABA on performance, carcass characteristics and serum parameters in broilers under circular heat stress, and reported that GABA feed intake significantly increased. Moreover, Jing et la. (2010) and Tan et al. (2012) reported that GABA can improve feed intake, reproduction and anti-stress. These reasons are due to GABA having an effect on the central nervous system, and the brain collecting information from special senses and receptors in the digestive tract wall and metabolizing tissues. This information is integrated and used to determine what food to eat. Thus, increased animal feed intake is also increased growth production. Optimal level for broilers feed intake was 2mg/kg diet. Zou et al. (2009) supplemented 10 mg/kg of GABA in the basal diet of growth finishing swine in hot summer. They reported that feed intake was not affected. Tao et al. (2010), who studied effects and mechanism of GABA on laying performance and egg quality of laying hens under high temperature in summer, also reported feed intake was not significantly affected by supplementing 25, 50, 75, 100 mg/kg GABA in the basal diet. This is due to GABA levels not having enough affect on animals because the studies were conducted under heat stress temperatures and with big experimental animals, otherwise D'Mello (2000) reported, if the animals are already well fed and managed so that the rate of production of meat milk or eggs is already optimal, then to stimulate intake would only lead to more deposition of fat, which is not required in modern animal production. It's a good idea to pursue this line of thinking in a follow-up study. However, based on the results, the optimal level of GABA from germinated paddy rice for broiler feed intake was 2 mg/kg diet.

Table 4.3 Effect GABA levels on feed intake in broilers (g/h/d)

Week	Level of GABA, mg/kg					SEM
Feed intake	0.00	0.50	1.00	1.50	2.00	
W1	21.07	21.4	22.26	20.84	21.15	0.29
W2	59.65	60.71	61.53	60.24	61.81	0.45
W3	99.65	100.00	98.42	100.56	100.77	1.34
W1-W3	60.11	60.95	60.73	60.54	61.24	0.53
W4	135.36	139.65	124.24	134.09	132.15	2.92
W5	163.27[b]	164.89[b]	137.16[c]	159.69[b]	189.88[a]	4.76
W6	189.02	192.12	199.82	163.50	173.83	6.3
W4-W6	162.54	165.55	153.73	152.42	165.28	2.97
W1-W6	111.33	113.25	107.23	106.48	113.26	1.63

[a, b] Means in the same row with different superscripts are significantly different (P<0.05)

4.3 Effect of GABA levels on average daily gain in broilers

The effect of GABA from germinated rice on the average daily gain is presented in Table 4.4. The effect of GABA levels on average daily gain in weeks 1, 3, 4, and 5 was not significantly different (P>0.05), while week 2 was significantly different (P<0.05) at 38.57, 40.35, 40.03, 40.85, and 43.37 g/h/d. The treatment groups that were supplemented levels of GABA were 0, 0.5, 1, 1.5 and 2 mg/kg of the diet respectively. The treatment that GABA supplemented at 2 mg/kg of the diet was higher than other treatment groups. Average daily gain was 43.37 g/h/d. On the other hand, the 6^{th} week was significantly different (P<0.05). Average daily gain was 87.3, 86.67, 109.09, 70.81, and 79.93 g/h/d in the treatment groups that had supplemented levels of GABA 0, 0.5, 1, 1.5 and 2 mg/kg of diet respectively, Treatment that GABA supplemented at 1 mg/kg of the diet was higher than other treatment groups.
The average daily gain was 109.09. This result was according to Chen et al. (2001), who studied the effect of GABA on heat stress broilers, and reported a significant increase in average daily gain. In addition, Zou et al. (2009) supplemented GABA in the basal diet of growth finishing swine under heat stress, and reported a significant increase in average daily gain . Jaicheng et al. (2008) reported that supplemented GABA with a basal diet can increase average daily gain in pigs. Zhang et al. (2012), who studied effect of dietary GABA in laying performance, egg quality, immune activity and endocrine hormone in heat stress Roman hens, reported that egg production increased. Xie et al. (2012) also reported that average daily gain was significantly increased by supplemented 80 mg/kg GABA in the basal diet of chickens under heat stress. As a result, many reports show that GABA affected average daily gain in animals under heat stress in accordance with the current study that shows supplementary GABA with a basal diet affected an increase in average daily gain. The increase is due to increasing the feed intake, the central nervous system’s control of all the secreting hormones in the body, and the thyroid hormone (T3 and T4). The thyroid hormone is related to the metabolic system..

Table 4.4 Effect of GABA levels on average daily gain in broilers (g/h/d)

Week	Level of GABA, mg/kg					SEM
ADG	0.00	0.50	1.00	1.50	2.00	
W1	17.53	17.42	17.44	16.81	17.17	0.25
W2	38.57[b]	40.35[ab]	40.03[ab]	40.85[ab]	43.37[a]	0.59
W3	67.86	66.43	66.07	68.93	68.42	1.02
W1-W3	41.320	41.403	41.183	42.200	42.985	0.47
W4	83.57	81.79	78.02	77.54	81.43	1.65
W5	62.04	68.20	57.91	62.21	71.10	4.17
W6	87.3[ab]	86.67[ab]	109.09[a]	70.81[b]	79.93[ab]	5.35
W4-W6	77.638	78.888	81.675	70.185	77.485	2.09
W1-W6	59.47	60.14	61.42	56.19	60.23	1.11

[a, b] Means in the same row with different superscripts are significantly different (P<0.05)

4.4 Effect of GABA Levels on Feed Conversion Ratio in Broilers

Effect of GABA from germinated paddy rice on feed conversion ratio is presented in Table 4.5. The effect of GABA levels on the feed conversion ratio every week were not significant (P>0.05). These results are in disagreement with Chen et al. (2001) who studied the effect of GABA on the heat stress broilers and reported a significantly decreased feed conversion ratio. Zhigang et al. (2013) who studied the effect of GABA on performance parameters and some plasma metabolites in cherry valley ducks under high ambient temperature showed that feed conversion ratio decreased. Otherwise, Zhang et al. (2012) who studied the effect of dietary GABA on laying performance, egg quality, immune activity and endocrine hormone in heat stress Roman hens reported an increase in feed egg ratio. Zou et al. (2009) supplemented 10 mg/kg of GABA in the basal diet of growth finishing swine during the hot simmer and reported an increased feed conversion ratio. Dai et al. (2011) who studied the effects of dietary glutamate and GABA on performance, carcass characteristics and serum parameters in broilers under circular heat stress, reported that GABA affected a feed conversion ratio that significantly increased. Consequently GABA had effect on the feed conversion ratio of poultry, but in the current study it was not significant because the GABA content was not sufficient enough to effect it.

Table 4.5 Effect of GABA levels on feed conversion ratio in broilers

Week	Level of GABA, mg/kg					SEM
FCR	0.00	0.50	1.00	1.50	2.00	
W1	1.21	1.28	1.28	1.25	1.24	0.02
W2	1.55	1.51	1.55	1.48	1.43	0.02
W3	1.47	1.51	1.49	1.46	1.48	0.01
W1-W3	1.45	1.47	1.47	1.43	1.42	0.01
W4	1.62	1.71	1.59	1.73	1.63	0.03
W5	2.87	2.70	2.64	2.70	2.80	0.19
W6	2.20	2.40	1.85	2.37	2.22	0.10
W4-W6	2.10	2.14	1.88	2.18	2.13	0.05
W1-W6	1.87	1.90	1.74	1.89	1.88	0.03

4.5 Effect of GABA Levels on Carcass Characteristics in Broilers

The effect of GABA from germinated paddy rice on carcass percentages is presented in Table 4.6. The result show effects to the eviscerated carcass, breast muscle, drumstick, abdominal fat, wing, liver, spleen, and heart percentage were not significant ($P>0.05$). The dressed carcass percentage was significantly high ($P<0.05$) in the treatment that supplemented 1 mg/kg of GABA. The effects of different levels of GABA on carcass percentage increased only the dressed carcass percentage while Dai et al. (2011) reported the effects of dietary GABA on performance, carcass characteristics and serum parameters in broilers under circular heat stress increased the eviscerated carcass, breast muscle, and abdominal fat percentage. The thigh and gizzard percentages are presented in Table 4.6. The control group was higher than the experimental groups ($P<0.05$). However, the results show that the GABA level from germinated paddy rice affected carcass characteristics in broilers. These results were consistent with Dai et al. (2012) who reported GABA effects on carcass yield in broilers under heat stress feeding. Moreover, Xiao-ting et al. (2009) also reported that GABA effected carcass characteristics and enhanced thyroid function in growth finishing swine. Shichang et al. (2007) reported that GABA affected a thyroid hormone decrease and an increase in concentrate of glucose and corticosteroid. GABA from germinated paddy rice affected carcass quality in broilers. The level of GABA in this study was not enough to affect all the carcass characteristics. In any follow up study GABA levels in the diet should be increased.

Table 4.6 Effect of GABA levels on carcass characteristics in broilers

Parameters	Level of GABA, mg/kg					SEM
	0.00	0.50	1.00	1.50	2.00	
Dressed %	93.31[ab]	93.00[ab]	91.81[b]	92.99[ab]	94.91[a]	0.38
Eviscerated %	80.72	80.77	81.87	81.18	85.14	0.86
Breast %	22.93	23.78	24.12	22.6	21.63	0.42
Thigh %	13.51[ab]	14.16[ab]	15.09[a]	13.08[ab]	12.79[b]	0.32
Drumstick%	11.60	11.66	11.38	11.38	10.66	0.16
Abdominal%	1.15	1.21	0.95	0.94	1.14	0.06
Wing%	8.49	8.62	8.38	8.54	8.15	0.13
Gizzard%	2.25[a]	2.04[ab]	2.01[ab]	2.0[ab]	1.83[b]	0.05
Liver%	2.22	2.56	2.12	2.42	2.35	0.07
Spleen%	0.20	0.24	0.15	0.20	0.16	0.02
Heart%	0.56	0.62	0.6	0.58	0.57	0.01

[a, b] Means in the same row with different superscripts are significantly different ($P<0.05$)

4.6 Effect of GABA Levels on Hematological Value in Broilers

The effects of the GABA on hematological value are shown in Table 4.7. Total white blood cell count, total red blood cell count, basophils, monocytes, lymphocytes and heterophils were not significantly different ($P>0.05$) within treatment groups. The results indicated that the GABA in the diets had not affected hematological value. While, PCV and eosinophils were significantly different (P<0.0%) they were within a normal range when compared with the reference values of Nowaczewski et al. (2012); therefore all broilers in this experiment were healthy. GABA activities might reduce activity of serum GPT and the level of plasma T4 but increase the levels of T3 in broilers (Jianzhen et al., 2010), increased T3 and T4 in pigs (Xu et al., 2009), increased lymphocytes in chickens (Xie et al., 2012), increased serum glutathione peroxidase and superoxide dismutase in cows (Wang et al., 2013), improved secretion of growth hormone melatonin and thyroid stimulating hormone in pigs (Fan et al., 2007). GABA could promote laying performance by regulating endocrine, enhancing the immunity and antioxidant capacity in pigeons (Huang et al., 2011), increasing antioxidant capacity, immune function, and increasing the secretion of gonadal and thyroid hormones (Tao et al., 2010). In contrast, Jiacheng et al. (2008) revealed that 10mg/kg GABA supplemented in diets had no effect on the concentration of T4 and FT4 in swine. Consequently, GABA levels in the diet revealed a positive effect on hematological values in broilers. This study indicated that the broilers were healthy.

Table 4.7 Effect of GABA levels on hematological value in broilers

Parameter	Level of GABA, mg/kg					SEM
	0.00	0.50	1.00	1.50	2.00	
PCV (%)	37.25[a]	38.62[a]	37.87[a]	30.62[b]	30.00[b]	1.07
RBC ($x10^6$ cell/µl)	2.63	2.61	2.51	2.53	2.45	0.05
WBC ($x10^4$ cell/µl)	2.07	1.97	2.10	2.14	2.07	0.04
Typical of WBC						
Eosinophils (%)	1.37[b]	3.12[a]	2.75[ab]	2.62[ab]	2.62[ab]	0.24
Basophils (%)	1.87	1.12	1.37	1.25	1.87	0.21
Monocytes (%)	1.37	0.62	0.75	0.87	0.62	0.12
Lymphocytes (%)	59.62	57.87	61.00	58.75	60.75	0.98
Heterophils (%)	35.75	37.25	34.12	36.50	35.37	0.97

[a, b] Means in the same row with different superscripts are significantly different (P<0.05)

4.7 Effect of GABA Levels on Economic Benefits Return in Broilers

The feed costs during the experimental period (February until March, 2014) are presented in Table 4.8. They show that the feed price was 19.54 baht per kilogram for a broiler starter diet and 17.98 baht per kilogram for broiler finisher diet, that prices were lower than reported by the Thai office of agricultural economics (2014). Their price was 22.26 baht per kilogram. The broiler body weight gains overall in the experimental period were around 2,360.00 to 2,529.90g. Feed intake during the experimental period was around 4472.40 g to 4757.10g. While the price of live broilers was 45 baht per kilogram within the March 2014 in Mahasarakham province Thailand, they were higher than reported by Thai feed mill association (2014) who found their price at 40.63 baht per kilogram. The finishing broilers price was not significantly different 112.41, 113.67, 116.1, 106.2 and 113.84 baht per head within the group that supplemented of GABA level 0, 0.5, 1, 1.5 and 2 mg/kg, respectively. Therefore, GABA levels from germinated paddy rice did not affect the economic benefit return in broilers due to body weight gain and feed intake being not significantly different.

Table 4.8 Effect of GABA levels on economic benefits return

Items	GABA levels in diet (mg/kg)					SEM
	0.00	0.50	1.00	1.50	2.00	
BWG (g/h)	2498.10	2526.10	2580.00	2360.00	2529.90	46.77
FI (g/h)	4676.00	4756.60	4503.90	4472.40	4757.10	68.66
Feed conversion ratio						
W1-W3	1.45	1.47	1.47	1.43	1.42	0.01
W4-W6	2.10	2.14	1.88	2.18	2.13	0.05
W1-W6	1.87	1.90	1.74	1.89	1.88	0.03
Feed cost (baht/kg)						
W1-W3	19.54	19.54	19.54	19.54	19.54	-
W4-W6	17.98	17.98	17.98	17.98	17.98	-
Feed cost per gain (FCG, baht/h)						
W1-W3	24.66	25.01	24.92	24.84	25.13	0.22
W3-W6	61.37	62.50	58.04	57.55	62.40	1.12
W1-W6	86.04	87.52	82.96	82.39	87.54	1.25
SBR (baht/h)	112.41	113.67	116.10	106.20	113.84	-
NPR (baht/h)	26.37	26.15	33.14	23.81	26.30	-
ROI	0.00	-0.22	+6.77	-2.56	-0.07	-
Production index	270.96	241.42	297.48	223.28	2305.8	118.56
Survival percentage	85	77.5	85	75	72.5	3.40

BWG=body weight gain overall experimental period gram per head, FI=feed intake overall experimental period gram per head, SBR = Salable bird return, NPR = Net profits return per bird, ROI = Return of investment

CHAPTER 5

Conclusion and Suggestion

5.1 Conclusion

This study aims to investigate the effect of different levels of GABA from germinated paddy rice in a basal diet on growth performance and carcass characteristics in broilers.

5.1.1 Chemical composition of diet

The chemical composition of the diet in both, starter and finisher was similar to the NRC (1994). The GABA content in germinated paddy rice (229.30 mg/kg) was analyzed by high performance liquid chromatography.

5.1.2 Effect of GABA levels on growth performance in broilers

The effect of GABA levels on feed intake was significantly high in treatment groups that supplemented with GABA at 2 mg/kg in the 5th week at 189.88 gram per head per day. The average daily gain was significantly high in the treatment group that supplemented with GABA 2 mg/kg. Daily gain in week two, and week six average daily gain, was high in the treatment group that supplemented 1.5 mg/kg diet. Feed conversion ratio was not significant in all treatment groups due to GABA levels not being high enough to affect in broilers.

5.1.3 Effect of GABA levels on carcass characteristics in broilers

The eviscerated carcass, breast muscle, drumstick, abdominal fat, wing, liver, spleen, and heart percentage were not affected by the GABA supplement, while the dressed carcass percentage was highly affected by the GABA supplement at 2 mg/kg of GABA. The thigh was high in the supplemented group of GABA levels 1 mg/kg.

5.1.4 Effect of GABA levels on hematological values in broilers

The effects of the GABA on hematological values, white blood cell count, total red blood cell count, basophils, monocytes, lymphocytes and heterophils were not significantly different ($P>0.05$) between treatments. The results indicate that the GABA in the diets had no effect on hematological values, while, hematocrit and eosinophils was significantly different (P<0.05%) but within a normal range when compared with the reference values of Nowaczewski et al. (2012). Therefore all broilers in this experiment are healthy.

5.2 Suggestion

1. Should be increased GABA content from germinated paddy rice supplementation in basal diet due to the results of this study not clearly appeared

2. Should be tried to find more sources of GABA from natural sources to supplementation in basal diet of broilers.

3. Should be study effect of increase feed intake on deposition of fat in broiler. Due to requirement of modern animal production is low fat meat.

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APPENDICES

Appendix

Analysis of variance (ANOVA)

Appendix table1 Effect of GABA levels on feed intake (g/h/d)

SOV	df	SS	MS	F	P
W1					
Treatment	4	6.91	1.72	1.06	0.40
Error	15	24.43	1.62		
W2					
Treatment	4	12.79	3.19	0.76	0.56
Error	15	62.95	4.19		
W3					
Treatment	4	13.83	3.45	0.08	0.98
Error	15	664.44	44.29		
W4					
Treatment	4	513.63	128.40	0.71	0.60
Error	15	2730.25	182.01		
W5					
Treatment	4	5620.35	1405.08	7.01	0.002
Error	15	3007.17	200.47		
W6					
Treatment	4	3458.33	864.58	1.12	0.38
Error	15	11621.57	774.77		
W1-W6					
Treatment	4	170.08	42.52	0.75	0.57
Error	15	845.72	56.38		

Appendix table2 Effect of GABA levels on average daily gain (g/h/d)

SOV	df	SS	MS	F	P
W1					
Treatment	4	1.38	0.34	0.23	0.91
Error	15	22.72	1.51		
W2					
Treatment	4	48.90	12.22	2.16	0.12
Error	15	84.9	5.66		
W3					
Treatment	4	24.80	6.20	0.25	0.90
Error	15	370.10	24.67		
W4					
Treatment	4	107.40	26.85	0.44	0.78
Error	15	923.53	61.56		
W5					
Treatment	4	447.32	111.83	0.27	0.89
Error	15	6153.94	410.26		
W6					
Treatment	4	3200.38	800.09	1.56	0.23
Error	15	7688.28	512.55		
W1-W6					
Treatment	4	62.44	15.61	0.57	0.68
Error	15	408.89	27.25		

Appendix table3 Effect of GABA levels on feed conversion ratio

SOV	df	SS	MS	F	P
W1					
Treatment	4	0.01	0.003	0.25	0.90
Error	15	0.19	0.01		
W2					
Treatment	4	0.04	0.01	1.38	0.28
Error	15	0.11	0.007		
W3					
Treatment	4	0.005	0.001	0.27	0.89
Error	15	0.07	0.005		
W4					
Treatment	4	0.06	0.01	1.23	0.34
Error	15	0.18	0.01		
W5					
Treatment	4	0.13	0.03	0.04	0.99
Error	15	13.72	0.91		
W6					
Treatment	4	3.62	0.90	1.98	0.14
Error	15	6.87	0.45		
W1-W6					
Treatment	4	0.06	0.01	1.04	0.41
Error	15	0.24	0.01		

Appendix table4 Effect of GABA levels on carcass characteristics

SOV	df	SS	MS	F	P
Dressed (%)					
Treatment	4	19.82	4.95	2.19	0.11
Error	15	33.96	2.26		
Eviscerated (%)					
Treatment	4	54.71	13.67	0.91	0.48
Error	15	224.79	14.98		
Breast (%)					
Treatment	4	15.51	3.87	1.15	0.37
Error	15	50.80	3.38		
Thigh (%)					
Treatment	4	13.45	3.36	1.93	0.15
Error	15	26.16	1.74		
Drumstick (%)					
Treatment	4	2.56	0.64	1.29	0.31
Error	15	7.44	0.49		
Abdominal fat (%)					
Treatment	4	0.25	0.06	0.83	0.52
Error	15	1.13	0.07		
Wing (%)					
Treatment	4	0.54	0.13	0.35	0.84
Error	15	5.88	0.39		
Gizzard (%)					
Treatment	4	0.35	0.08	2.82	0.06
Error	15	0.46	0.03		
Liver (%)					
Treatment	4	0.46	0.11	1.29	0.31
Error	15	1.34	0.08		
Spleen (%)					
Treatment	4	0.02	0.005	0.93	0.47
Error	15	0.08	0.005		
Heart (%)					
Treatment	4	0.009	0.002	0.49	0.74
Error	15	0.07	0.004		

Appendix table5 Effect of GABA levels on hematological value

SOV	df	SS	MS	F	P
PCV (%)					
Treatment	4	282.12	70.53	6.90	0.002
Error	15	153.31	10.22		
WBC (104 cell/µl)					
Treatment	4	0.05	0.01	0.32	0.86
Error	15	0.66	0.04		
RBC (106 cell/µl)					
Treatment	4	0.08	0.02	0.36	0.83
Error	15	0.92	0.06		
Eosinophils (%)					
Treatment	4	7.00	1.75	1.81	0.17
Error	15	14.50	0.96		
Basophils (%)					
Treatment	4	2.00	0.50	0.54	0.71
Error	15	14.00	0.93		
Moncytes (%)					
Treatment	4	1.55	0.38	1.66	0.21
Error	15	3.50	0.23		
Lymphocytes (%)					
Treatment	4	27.92	6.98	0.31	0.86
Error	15	339.37	22.62		
Heterophils (%)					
Treatment	4	22.32	5.58	0.25	0.90
Error	15	332.87	22.19		

BIOGRAPHY

BIOGRAPHY

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